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<b>(21) International Application Number:</b> PCT/US00/07661 <b>(22) International Filing Date:</b> 23 March 2000 (23.03.00) <b>(30) Priority Data:</b> 60/126,504                      26 March 1999 (26.03.99)      US 60/174,847                      7 January 2000 (07.01.00)      US <b>(71) Applicant (for all designated States except US):</b> HUMAN GENOME SCIENCES, INC. [US/US]; 9410 Key West Avenue, Rockville, MD 20850 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ROSEN, Craig, A. [US/US]; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US). RUBEN, Steven, M. [US/US]; 18528 Heritage Hills Drive, Laytonsville, MD 20882 (US). KOMAT-SOULIS, George [US/US]; 9518 Garwood Street, Silver Spring, MD 20901 (US). <b>(74) Agents:</b> HOOVER, Kenley, K. et al.; Human Genome Sciences, Inc., 9410 Key West Avenue, Rockville, MD 20850 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> 45 HUMAN SECRETED PROTEINS		
<b>(57) Abstract</b> <p>The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to these novel human secreted proteins.</p>		

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## 45 Human Secreted Proteins

### *Field of the Invention*

This invention relates to newly identified polynucleotides and the polypeptides encoded by these polynucleotides, uses of such polynucleotides and polypeptides, and their production.

### *Background of the Invention*

Unlike bacterium, which exist as a single compartment surrounded by a membrane, human cells and other eucaryotes are subdivided by membranes into many functionally distinct compartments. Each membrane-bounded compartment, or organelle, contains different proteins essential for the function of the organelle. The cell uses "sorting signals," which are amino acid motifs located within the protein, to target proteins to particular cellular organelles.

One type of sorting signal, called a signal sequence, a signal peptide, or a leader sequence, directs a class of proteins to an organelle called the endoplasmic reticulum (ER). The ER separates the membrane-bounded proteins from all other types of proteins. Once localized to the ER, both groups of proteins can be further directed to another organelle called the Golgi apparatus. Here, the Golgi distributes the proteins to vesicles, including secretory vesicles, the cell membrane, lysosomes, and the other organelles.

Proteins targeted to the ER by a signal sequence can be released into the extracellular space as a secreted protein. For example, vesicles containing secreted proteins can fuse with the cell membrane and release their contents into the extracellular space - a process called exocytosis. Exocytosis can occur constitutively or after receipt of a triggering signal. In the latter case, the proteins are stored in secretory vesicles (or secretory granules) until exocytosis is triggered. Similarly, proteins residing on the cell membrane can also be secreted into the extracellular space by proteolytic cleavage of a "linker" holding the protein to the membrane.

Despite the great progress made in recent years, only a small number of genes encoding human secreted proteins have been identified. These secreted proteins include the commercially valuable human insulin, interferon, Factor VIII, human

growth hormone, tissue plasminogen activator, and erythropoietin. Thus, in light of the pervasive role of secreted proteins in human physiology, a need exists for identifying and characterizing novel human secreted proteins and the genes that encode them. This knowledge will allow one to detect, to treat, and to prevent  
5 medical diseases, disorders, and/or conditions by using secreted proteins or the genes that encode them.

### *Summary of the Invention*

The present invention relates to novel polynucleotides and the encoded  
10 polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The  
15 invention further relates to screening methods for identifying binding partners of the polypeptides.

### *Detailed Description*

#### Definitions

20 The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated  
25 polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by  
30 electrophoresis and transferred onto blots), sheared whole cell genomic DNA



preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO:X was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table 1, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M  $\text{NaH}_2\text{PO}_4$ ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 µg/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress

background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above,

5 due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically 10 any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of 15 single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of 20 triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces 25 chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as 30 posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more

detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, **PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES**, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); **POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS**, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth Enzymol* 182:626-646 (1990); Rattan et al., *Ann NY Acad Sci* 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences identified by an integer specified in Table 1.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar

to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

Many proteins (and translated DNA sequences) contain regions where the amino acid composition is highly biased toward a small subset of the available residues. For example, membrane spanning domains and signal peptides (which are also membrane spanning) typically contain long stretches where Leucine (L), Valine (V), Alanine (A), and Isoleucine (I) predominate. Poly-Adenosine tracts (polyA) at the end of cDNAs appear in forward translations as poly-Lysine (poly-K) and poly-Phenylalanine (poly-F) when the reverse complement is translated. These regions are often referred to as "low complexity" regions.

Such regions can cause database similarity search programs such as BLAST to find high-scoring sequence matches that do not imply true homology. The problem is exacerbated by the fact that most weight matrices (used to score the alignments generated by BLAST) give a match between any of a group of hydrophobic amino acids (L, V and I) that are commonly found in certain low complexity regions almost as high a score as for exact matches.

In order to compensate for this, BLASTX.2 (version 2.0a5MP-WashU) employs two filters ("seg" and "xnu") which "mask" the low complexity regions in a particular sequence. These filters parse the sequence for such regions, and create a new sequence in which the amino acids in the low complexity region have been replaced with the character "X". This is then used as the input sequence (sometimes referred to herein as "Query" and/or "Q") to the BLASTX program. While this regime helps to ensure that high-scoring matches represent true homology, there is a negative consequence in that the BLASTX program uses the query sequence that has been masked by the filters to draw alignments.

Thus, a stretch of "X"s in an alignment shown in the following application does not necessarily indicate that either the underlying DNA sequence or the translated protein sequence is unknown or uncertain. Nor is the presence of such

stretches meant to indicate that the sequence is identical or not identical to the sequence disclosed in the alignment of the present invention. Such stretches may simply indicate that the BLASTX program masked amino acids in that region due to the detection of a low complexity region, as defined above. In all cases, the reference sequence(s) (sometimes referred to herein as "Subject", "Sbjct", and/or "S") indicated in the specification, sequence table (Table 1), and/or the deposited clone is (are) the definitive embodiment(s) of the present invention, and should not be construed as limiting the present invention to the partial sequence shown in an alignment, unless specifically noted otherwise herein.

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### **Polynucleotides and Polypeptides of the Invention**

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 1**

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Fetal Kidney; NCI\_CGAP\_Pr2; and Activated T-Cell (12hs)/Thiouridine labelled Eco.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:11 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1426 of SEQ ID NO:11, b is an integer of 15 to 1440, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 2**

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting

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example, the sequence accessible through the following database accession no.  
 gn1|PID|e234798 (all information available through the recited accession number is  
 incorporated herein by reference) which is described therein as "R11A8.7  
 [Caenorhabditis elegans]." A partial alignment demonstrating the observed homology  
 is shown immediately below.

```

>gn1|PID|e234798 R11A8.7 [Caenorhabditis elegans]
Length = 1525

10      Plus Strand HSPs:
Score = 392 (138.0 bits), Expect = 2.5e-34, P = 2.5e-34
Identities = 105/293 (35%), Positives = 162/293 (55%), Frame = +3

15      Q:  417 EVLRRLTSSVSCALDEAAALTRMRAESTANAGQSDNLSLAECSEGDVNAVRLKLLIDGR 596
      E+ ++ +S + ++ +L +S GQ L SEGD +
      S:  78 EIQRKGTSSGTLISTSSKSLMAKSVQSQQQGQ-----LRRTHSEGD--GAERAKSRSN 130

20      Q:  597 SVNHETEG-ESLLCLACAGYYELAQVLLAHNANVEDRGIKGDITPLMAAANGHHVKIV 773
      ++++ TE E+ L +AC+ G+ ++ ++LL AN+E R KG +PL+ AA GH +V
      S:  131 AIDKATETTTLETPLTIACANGHKDIVEILLKEGANIEHRDKKG-FSPLIIAATAGHSSVV 189

25      Q:  774 KLLLAHKADVNAQSS-TGNTALTACAGGYVDVVKVLESCESTEDHNENGHGTPIMEAGS 950
      ++LL + A + AQS T +TAL+ AC+GG DUV++LL GA+ E N + +TPL A S
      S:  190 EVLLKNHAAIEAQSDRTKDTALSLACSGGRKDUVELLHAHGANKHEHRVSDYTPLSLASS 249

30      Q:  951 AGHVEVARLLLENGAGINTHS-NEFKESALTACYKGHEMVRFLLEAGADQEHKTD-EM 1124
      G++E+ +LL G+ IN+ + ++ S L LA GH E R LLE G+D ++
      S:  250 GGYIEIVNMLLTAGSEINSRTGSKLGISPLMLASMNGHREATRVLLEKGSDDINAIETNR 309

      Q:  1125 HTALMEACMDGHVEVARLLDSSGAQVNMPADSFEPLTLAACGGHVELAALLIERGA 1295
      +TAL A G EV +LL A V A + +PL A GG+V++ LLI GA
      S:  310 NTALTASFGQRTVEVKLLLAYNANVEHRAKTGLTPLMECASGGYVDGNLLIAAGA 366
  
```

The segment of gn1|PID|e234798 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 103.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 104 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

Additionally, the translation product of this gene shares sequence homology with ankyrin (see, e.g., Genbank accession gblAAB01605.1| ankyrin 3 [Mus musculus]: all references available through this accession are hereby incorporated by reference herein.) which are peripheral membrane proteins believed to interconnect

integral proteins with the spectrin-based membrane skeleton. Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with ankyrin and ankyrin-like proteins. Such activities are known in the art (e.g., Kordeli et al., J. Biol. Chem. 270 (5), 2352-2359 (1995)),

5 some of which are described elsewhere herein.

Preferred polypeptides comprise or alternatively consist of the following amino acid sequence:

MLETASKLLSGTADGADLRTVDPETQARLEALLEAAGIGKLSTADGKAFAD  
PEVLRRLTSSVSCALDEAAAALTRMRAESTANAGQSDNRS LAEACSEGDVNA  
10 VRKLLIEGRSVNEHTEEGESLLCLACSAGYYELAQVLLAMHANVEDRGIKGD  
ITPLMAAANGGHVKIVKLLLAHKADVNAQSTGNTALTYACAGGYDVVK  
VLLESGASIEDHNENGHTPLMEAGSAGHVEVARLLLENGAGINTHSNEFKES  
ALTLCYKGHLEMVRFLEAGADQEHKTDDEMHTALMEACMDGHVEVARLL  
LD SG AQVNMPADSFESPLTLAACGGHVELAALLIERGASLEEVDNDEGYTPLM  
15 EA (SEQ ID NO: ) Polynucleotides encoding these polypeptides are also  
encompassed by the invention. Antibodies that bind to polypeptides of the invention  
are also encompassed in the invention.

It has been discovered that this gene is expressed primarily in the following  
tissues/cDNA libraries: HUMAN TONSILS. FRACTION 2; HSC172 cells; and

20 Smooth muscle, control.

Many polynucleotide sequences, such as EST sequences, are publicly  
available and accessible through sequence databases. Some of these sequences are  
related to SEQ ID NO:12 and may have been publicly available prior to conception of  
the present invention. Preferably, such related polynucleotides are specifically  
25 excluded from the scope of the present invention. To list every related sequence  
would be cumbersome. Accordingly, preferably excluded from the present invention  
are one or more polynucleotides comprising a nucleotide sequence described by the  
general formula of a-b, where a is any integer between 1 to 1330 of SEQ ID NO:12. b  
is an integer of 15 to 1344. where both a and b correspond to the positions of  
30 nucleotide residues shown in SEQ ID NO:12. and where b is greater than or equal to a  
+ 14.



# **FEATURES OF PROTEIN ENCODED BY GENE NO: 3**

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in

- 5 Soares placenta Nb2HP; Soares\_senescent\_fibroblasts\_NbHSF;  
Soares\_NhHMPu\_S1; Soares\_NhHMPu\_S1; Soares\_NFL\_T\_GBC\_S1;  
NCL\_CGAP\_Brn23; Primary Dendritic Cells, lib 1; Soares infant brain 1NIB;  
Soares\_senescent\_fibroblasts\_NbHSF; Soares\_fetal\_liver\_spleen\_1NFLS\_S1;  
Human umbilical vein endothelial cells, IL-4 induced; Stratagene lung (#937210);
- 10 NCL\_CGAP\_Co12; NCL\_CGAP\_GCB1; Soares melanocyte 2NbHM;  
Soares\_multiple\_sclerosis\_2NbHMSP; Human (Caco-2) cell line, adenocarcinoma,  
colon, remake; Human colon carcinoma (HCC) cell line, remake; HUMAN  
STOMACH; Smooth Muscle Serum Treated, Norm; Soares adult brain N2b4HB55Y;  
NTERA2 + retinoic acid, 14 days; Human Osteoclastoma, re-excision; TF-1 Cell Line
- 15 GM-CSF Treated; L428; Stromal cell TF274; Soares\_testis\_NHT;  
Soares\_multiple\_sclerosis\_2NbHMSP; NCI\_CGAP\_Co10; Hepatocellular Tumor, re-  
excision; Colon Carcinoma; Smooth muscle, serum treated; Bone marrow; Human  
Osteoclastoma; Human Amygdala; Monocyte activated; Human 8 Week Whole  
Embryo and NCI\_CGAP\_Pr23.
- 20 Many polynucleotide sequences, such as EST sequences, are publicly  
available and accessible through sequence databases. Some of these sequences are  
related to SEQ ID NO:13 and may have been publicly available prior to conception of  
the present invention. Preferably, such related polynucleotides are specifically  
excluded from the scope of the present invention. To list every related sequence
- 25 would be cumbersome. Accordingly, preferably excluded from the present invention  
are one or more polynucleotides comprising a nucleotide sequence described by the  
general formula of a-b, where a is any integer between 1 to 3093 of SEQ ID NO:13, b  
is an integer of 15 to 3107, where both a and b correspond to the positions of  
nucleotide residues shown in SEQ ID NO:13, and where b is greater than or equal to a
- 30 + 14.

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## FEATURES OF PROTEIN ENCODED BY GENE NO: 4

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no.

- 5 gi|386924 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "MHC HLA-DQ-alpha chain precursor old gene name 'HLA-DQA' [Homo sapiens]." A partial alignment demonstrating the observed homology is shown immediately below.

```

10  >gi|386924 MHC HLA-DQ-alpha chain precursor old gene name 'HLA-DQA' [Homo
    old sapiens] >pir|I54444|I54444 MHC HLA-DQ-alpha chain precursor
    CLASS gene name 'HLA-DQA' - human (fragment) >sp|Q30086|Q30086 MHC
15  II HLA-DQ-ALPHA (DR2-DQW1/DR4 DQW3) (FRAGMENT). (SUB 2-233)
    >gi|619810 MHC class II HLA-DQ-alpha chain [Homo sapiens] (SUB
    {SUB 2-228) >gi|187937 MHC class II DQ-alpha protein [Homo sapiens]
    {SUB 97-233} >gi|553264 cell surface glycoprotein [Homo sapiens]
20  {SUB 7-88} >pir|H34513|H34513 MHC class II histocompatibility
    antigen DQ alpha-1.2 chain - gorilla (fragment) (SUB 19-81)
    Length = 233
25  Plus Strand HSPs:
    Score = 502 (176.7 bits), Expect = 2.4e-113, Sum P(4) = 2.4e-113
    Identities = 93/96 (96%), Positives = 94/96 (97%), Frame = +1
30  Q: 1348 TTEVPEVTVFSKSPVTLQQPNTLICLVADNIFPPVNNITWLSNGHAVTEGVSETSFLSKSD 1527
    T EVPEVTVFSKSPVTLQQPNTLICLVADNIFPPVNNITWLSNG +VTEGVSETSFLSKSD
    S: 87 TNEVPEVTVFSKSPVTLQQPNTLICLVADNIFPPVNNITWLSNGQSGVTEGVSETSFLSKSD 146
35  Q: 1528 HSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKH 1635
    HSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKH
    S: 147 HSFFKISYLTFLPSADEIYDCKVEHWGLDQPLLKH 182
40  Score = 451 (158.8 bits), Expect = 2.4e-113, Sum P(4) = 2.4e-113
    Identities = 81/83 (97%), Positives = 83/83 (100%), Frame = +1
    Q: 694 ADEHVASCGLNLYQFYGPSGQFTHEFDGDEQFYVDLEKKETANRNPEFSKFGGFDPPQGLR 873
    ADHVASCGLNLYQFYGPSGQ+THEFDGDEQFYVDLE+KETANRNPEFSKFGGFDPPQGLR
45  S: 6 ADHVASCGLNLYQFYGPSGQYTHEFDGDEQFYVDLERKETANRNPEFSKFGGFDPPQGLR 65
    Q: 874 NNAVAKHNLNIMIKRYNSTAATN 942
    NNAVAKHNLNIMIKRYNSTAATN
    S: 66 NNAVAKHNLNIMIKRYNSTAATN 88
50  Score = 142 (50.0 bits), Expect = 2.4e-113, Sum P(4) = 2.4e-113
    Identities = 23/36 (80%), Positives = 31/36 (86%), Frame = +3
    Q: 1962 LCPLGLMASIGIVVGTFTIIQGLRSVGASRHQGPL 2069
    +C LGL +GIVVGTFTIIQGLRSVGASRHQGPL
55  S: 198 VCALGLSVGLMEIVVGTFTIIQGLRSVGASRHQGPL 233

```

5 Q: 1882 WWSDLNFTSTEPEIPAPMSELTETVVCA 1968  
W D EPEIPAPMSELTETVVCA  
S: 172 WGLDQPLLKHWEPEIPAPMSELTETVVCA 200

The segments of g1386924 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 113, SEQ ID NO. 115, SEQ ID NO. 117 and SEQ ID NO. 119. Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

15 Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 114, SEQ ID NO. 116, SEQ ID NO. 118 and/or SEQ ID NO. 120 which correspond to the "Q" sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are  
20 also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Primary Dendritic Cells, lib 1 and to a lesser extent in Stratagene ovarian cancer (#937219); Primary Dendritic cells, frac 2; human tonsils; Human Adult Pulmonary; Stratagene lung (#937210); Human Fetal Heart; Soares 25 placenta Nb2HP; Soares\_pregnant\_uterus\_NbHPU; Human Pancreatic Carcinoma -- Screened; Human Spleen; Tongue carcinoma; H. Meningioma, M6; prostate-edited; Human Adult Spleen; Human Pancreatic Carcinoma; HUMAN STOMACH; Human Thyroid; Human T-cell lymphoma, re-excision; Soares retina N2b4HR; Cem cells cyclohexamide treated; Human Epididymus; H. Kidney Medulla, re-excision; Human 30 Thymus; L428; Ulcerative Colitis; Human Thymus; Human T-Cell Lymphoma; Soares breast 3NbHBst; Dendritic cells, pooled; NCI\_CGAP\_GC4; Colon Tumor II; Human Adult Pulmonary, re-excision; NCI\_CGAP\_Pr2; NCI\_CGAP\_Kid3; NCI\_CGAP\_Pr21; NCI\_CGAP\_Pr22; Colon Normal III; HUMAN B CELL

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LYMPHOMA; NCI\_CGAP\_GC3: Human Bone Marrow, treated; Hodgkin's  
Lymphoma II and Soares fetal liver spleen INFLS.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.  
59 as residues: Lys-32 to Lys-40. Polynucleotides encoding such polypeptides are  
5 also provided.

Many polynucleotide sequences, such as EST sequences, are publicly  
available and accessible through sequence databases. Some of these sequences are  
related to SEQ ID NO:14 and may have been publicly available prior to conception of  
the present invention. Preferably, such related polynucleotides are specifically  
10 excluded from the scope of the present invention. To list every related sequence  
would be cumbersome. Accordingly, preferably excluded from the present invention  
are one or more polynucleotides comprising a nucleotide sequence described by the  
general formula of a-b, where a is any integer between 1 to 2583 of SEQ ID NO:14, b  
is an integer of 15 to 2597, where both a and b correspond to the positions of  
15 nucleotide residues shown in SEQ ID NO:14, and where b is greater than or equal to a  
+ 14.

## FEATURES OF PROTEIN ENCODED BY GENE NO: 5

The computer algorithm BLASTX has been used to determine that the  
20 translation product of this gene shares sequence homology with, as a non-limiting  
example, the sequence accessible through the following database accession no.  
gnl|PIDd1035481 (all information available through the recited accession number is  
incorporated herein by reference) which is described therein as "KIAA0780 protein  
[Homo sapiens]." A partial alignment demonstrating the observed homology is shown  
25 immediately below.

```

>gnl|PIDd1035481 (AB019323) KIAA0780 protein [Homo sapiens]
>sp|D1035481|D1035481 KIAA0780 PROTEIN (FRAGMENT).
Length = 1100

Minus Strand HSPs:

Score = 313 (110.2 bits), Expect = 2.3e-38, Sum P(2) = 2.3e-38
Identities = 50/81 (61%), Positives = 63/81 (77%), Frame = -2
30
35 Q: 259 OGKCLQVHASCYGRPELVNEGWTCSRCAAHAWTAECCLCNLRGGALQMTTDRRWIHI 80

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15

C KCC+VHASCYGI + +GW C+RC +ANTAECCCLNLRGGAL+ T + +W HV+  
 S: 771 CAKCCVRVHASCYGIPSHCIDGWLCAKCKRNANTAECCCLNLRGGALKQTKNNKHAHV 830  
 Q: 79 CAIAVPEARFLNVIERHPVDI 17  
 CA+AVPE RF NV ER +D+  
 S: 831 CAVAVPEVRFTNVPERTQIDV 851  
 Score = 171 (60.2 bits), Expect = 2.8e-23, Sum P(2) = 2.8e-23  
 Identities = 46/132 (34%), Positives = 66/132 (50%), Frame = -3  
 Q: 525 EEVQRXARTEPYCAICTLFYVYQALQTEKEAPIASLGEGCPATLPKSRKTRPLIPE 346  
 +E + AR +P+CAICTL PY + + +E A + S+ KT+PLIPE  
 S: 665 QEYNATVARMPHCAICTLLMPYHKPDSNSEEND-ARWETKLEDEVVTSEG--RTKPLIPE 741  
 Q: 345 MCFYXGGENIEPLPANSYIGDDGTSPLIPAASAAACRSMPVAMASVPSWSMKAGRVPGAR 166  
 MCF EN E P N+++ +DGTS LI A C + + +PS + G+ AR  
 S: 742 MCFIYSEENIEYSPPNAFLEEDGTSLLISCACK-CVRVHASCYGIPSHCIDGWLCAK-ARC 799  
 Q: 155 TPGLRSAACATC 130  
 +A C C  
 S: 800 KRNANTAECCCL 811

The segments of gnlIPIDd1035481 that are shown as "S" above are set out in the sequence listing as SEQ ID NO. 125 and SEQ ID NO. 127.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 126 and/or SEQ ID NO. 128 which correspond to the Q sequences in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Fetal Brain, random primed; H. Normalized Fetal Liver, II; Colon Normal II; and HUMAN B CELL LYMPHOMA.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 60 as residues: Pro-18 to Gln-25, Ser-45 to Pro-50. Polynucleotides encoding such polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:15 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 804 of SEQ ID NO:15, b is an integer of 15 to 818, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.

5

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 6

It has been discovered that this gene is expressed primarily in Neutrophils IL-1 and LPS induced.

10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:16 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 289 of SEQ ID NO:16, b is an integer of 15 to 303, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a  
20 + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 7

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: T-Cell PHA 16 hrs; Neutrophils IL-1 and LPS induced.

25 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 62 as residues: Asp-6 to Gln-12. Polynucleotides encoding such polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are  
30 related to SEQ ID NO:17 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 919 of SEQ ID NO:17, b  
5 is an integer of 15 to 933, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 8

10 It has been discovered that this gene is expressed primarily in H. Atrophic Endometrium.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:18 and may have been publicly available prior to conception of  
15 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1090 of SEQ ID NO:18, b  
20 is an integer of 15 to 1104, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 9

25 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Pancreas Tumor and to a lesser extent in Primary Dendritic Cells, lib 1; Human Tonsil. Lib 3; H Umbilical Vein Endothelial Cells, frac A. re-excision; Hodgkin's Lymphoma I; HSC172 cells; H. Epididymus. caput & corpus; Amniotic Cells - TNF induced; Human T-cell lymphoma.re-excision: Human  
30 Tonsils. Lib 2: Human Amygdala.re-excision: STROMAL -OSTEOCLASTOMA: Human Umbilical Vein. Endo. remake: Human Adult Small Intestine: Human Infant

- Brain: Human Prostate: Soares\_fetal\_lung\_NbHL19W; Human Bone Marrow, re-excision; HUMAN JURKAT MEMBRANE BOUND POLYSOMES; Human Activated T-Cells; Human Heart; Human Activated Monocytes; Human Fetal Brain; Human Adrenal Gland Tumor: NTERA2. control; Human Placenta: Adipocytes;
- 5 Early Stage Human Brain; Primary Dendritic cells,frac 2; Human Fetal Heart; Human Adult Pulmonary,re-excision; Activated T-cell(12h)/Thiouridine-re-excision; Hodgkin's Lymphoma II: Human 8 Week Whole Embryo and Soares fetal liver spleen 1NFLS.

- Preferred epitopes include those comprising a sequence shown in SEQ ID NO.
- 10 64 as residues: Ala-5 to Leu-18. Polynucleotides encoding such polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:19 and may have been publicly available prior to conception of
- 15 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 811 of SEQ ID NO:19, b
- 20 is an integer of 15 to 825, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 10

- 25 It has been discovered that this gene is expressed primarily in Human Skin Tumor.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:20 and may have been publicly available prior to conception of
- 30 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence



would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1681 of SEQ ID NO:20, b is an integer of 15 to 1695, where both a and b correspond to the positions of  
5 nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 11

It has been discovered that this gene is expressed primarily in the following  
10 tissues/cDNA libraries: Human Pituitary, subt IX: Human Pituitary, subtracted; Soares infant brain 1NIB.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 66 as residues: Arg-22 to Trp-27, and/or Gln-29 to Glu-36. Polynucleotides encoding such polypeptides are also provided.

15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:21 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1161 of SEQ ID NO:21, b is an integer of 15 to 1175, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than or equal to a  
25 + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 12

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Stomach.re-excision: and human tonsils.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:22 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

5 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2006 of SEQ ID NO:22, b is an integer of 15 to 2020, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.

10

### FEATURES OF PROTEIN ENCODED BY GENE NO: 13

It has been discovered that this gene is expressed primarily in colon cancer , Human Umbilical Vein, Endo. remake, and brain.

Additionally, the translation product of this gene shares sequence homology

15 with METH-1 (see, e.g., Genbank accession AF060152\_1 and J. Biol. Chem. 274 (33), 23349-23357 (1999); all references available through this accession are hereby incorporated by reference herein.) which is a member of a family of proteins with angio-inhibitory activity, thus it is likely that polypeptides encompassed by the invention may exhibit antiangiogenic activity.

20 Based on the sequence similarity, the translation product of this clone is expected to share at least some biological activities with METH-1 and METH-1-like proteins. Such activities are known in the art (e.g., Vazquez et al., J. Biol. Chem. 274 (33), 23349-23357 (1999)), some of which are described elsewhere herein.

Preferred polypeptides comprise or alternatively consist of the following amino acid

25 sequence: NMPHDDN (SEQ ID NO: ), QHVMAPT (SEQ ID NO: ), HSHHCLIGFRFNMPHDDNNKCKEEGVKSPQHVMAPT LNFTNPWMWSKCS RKYTEFLE (SEQ ID NO: ) Polynucleotides encoding these polypeptides are also encompassed by the invention. Antibodies that bind to polypeptides of the invention are also encompassed in the invention, as well as fragments exhibiting biological

30 activity (e.g., antiangiogenesis).

Therefore, polynucleotides and polypeptides of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of diseases and conditions which include but are not limited to: colon and brain cancer. Similarly, polypeptides and antibodies directed to these polypeptides are useful in providing immunological probes for differential identification of the tissue(s) or cell type(s). For a number of disorders of the above tissues or cells, particularly of the neural tissue and clon. expression of this gene at significantly higher or lower levels may be routinely detected in certain tissues or cell types (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid and spinal fluid) or another tissue or sample taken from an individual having such a disorder, relative to the standard gene expression level, i.e., the expression level in healthy tissue or bodily fluid from an individual not having the disorder.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:23 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1813 of SEQ ID NO:23, b is an integer of 15 to 1827, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 14

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares melanocyte 2NbHM and to a lesser extent in NCL\_CGAP\_GCB1; Soares\_fetal\_heart\_NbHH19W; Soares infant brain 1NIB; Activated T-Cell (12hs)/Thiouridine labelled Eco; Soares fetal liver spleen 1NFLS; Stratagene colon (#937204); Soares\_placenta\_8to9weeks\_2NbHP8to9W; Osteoblasts;

- Larynx normal #10 261-273; Human T-cell lymphoma, re-excision: HSA 172 Cells; Soares\_NhHMPu\_S1; Synovial Fibroblasts (control); Stratagene fibroblast (#937212); Dendritic cells, pooled; CD34 positive cells (Cord Blood); NCL\_CGAP\_Br2; NCL\_CGAP\_Kid3; Soares placenta Nb2HP;
- 5 Soares\_senescent\_fibroblasts\_NbHSF; Human Microvascular Endothelial Cells, fract. B; Human OB MG63 control fraction I; Morton Fetal Cochlea; H. cerebellum, Enzyme subtracted; Mesangial cell, frac 2; Apoptotic T-cell, re-excision; NCL\_CGAP\_GCB1; Human endometrial stromal cells-treated with estradiol; Human Osteoclastoma Stromal Cells - unamplified; Human endometrial stromal cells; Jurkat
- 10 T-Cell, S phase; Human Ovary; human ovarian cancer; Human Osteoblasts II; Macrophage (GM-CSF treated); Human Liver, normal; Smooth muscle, serum induced, re-exc; Human T-Cell Lymphoma; Soares breast 3NbHBst; Adipocytes; Early Stage Human Brain; Soares\_multiple\_sclerosis\_2NbHMSP; Human Placenta; Endothelial-induced; Human Bone Marrow, treated; Human fetal heart, Lambda ZAP
- 15 Express; NCL\_CGAP\_AA1; NCL\_CGAP\_Co3; NCL\_CGAP\_Pr7; NCL\_CGAP\_Kid5; Keratinocyte; Human 8 Week Whole Embryo; Nine Week Old Early Stage Human; Human Cerebellum; Soares\_multiple\_sclerosis\_2NbHMSP and Primary Dendritic Cells, lib 1.

Many polynucleotide sequences, such as EST sequences, are publicly

20 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:24 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

25 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2009 of SEQ ID NO:24, b is an integer of 15 to 2023, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.

30

# FEATURES OF PROTEIN ENCODED BY GENE NO: 15

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Dendritic cells, pooled;

Soares\_multiple\_sclerosis\_2NbHMSP; and Human Fetal Lung III.

Many polynucleotide sequences, such as EST sequences, are publicly  
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:25 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention  
10 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 933 of SEQ ID NO:25, b is an integer of 15 to 947, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.

15

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 16

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Smooth muscle-ILb induced.

Many polynucleotide sequences, such as EST sequences, are publicly  
20 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:26 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention  
25 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1181 of SEQ ID NO:26, b is an integer of 15 to 1195, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.

30

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 17

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: breast lymph node CDNA library; Human Amygdala.re-excision; Human Chondrosarcoma; and Adipocytes.

Many polynucleotide sequences, such as EST sequences, are publicly  
5 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:27 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
10 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1633 of SEQ ID NO:27, b is an integer of 15 to 1647, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.

15

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 18**

It has been discovered that this gene is expressed primarily in Neutrophils IL-1 and LPS induced.

Many polynucleotide sequences, such as EST sequences, are publicly  
20 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:28 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
25 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2085 of SEQ ID NO:28, b is an integer of 15 to 2099, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 19**

It has been discovered that this gene is expressed primarily in Anergic T-cell.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:29 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 885 of SEQ ID NO:29, b is an integer of 15 to 899, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 20

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Soleus; and Human Cerebellum.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:30 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 695 of SEQ ID NO:30, b is an integer of 15 to 709, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 21

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with. as a non-limiting

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26

example, the sequence accessible through the following database accession no. gill1304599 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "ZNF127-Xp [Homo sapiens]." A partial alignment demonstrating the observed homology is shown

5 immediately below.

```

>gil1304599 ZNF127-Xp [Homo sapiens] >sp|Q13434|Q13434 ZNF127-XP.
      Length = 485

10      Plus Strand HSPs:

      Score = 398 (140.1 bits), Expect = 2.5e-36, P = 2.5e-36
      Identities = 73/89 (82%), Positives = 77/89 (86%), Frame = +3

15      Q:      6 EQKLILKYKEAMSNKACRYFDEGRGSCPPGGNCIFYKHAYPDGRRREEPQRQKVGTSRYRA 185
      +QKLI KYKEAMSNKAC+YFDEGRGSCPPG NCFYKH YPDGRRREEPQRQ+VGTSSR
      S:      389 KQKLIQKYKEAMSNKACKYFDEGRGSCPPGNCIFYKHMYPDGRREEPQRQVGTSSRNPG 448

20      Q:      186 QRRNHFWELIEERENSINPFIDEEEVVTF 272
      Q+RNFHWE EE NSINPF DEEE VTF
      S:      449 QQRNHFWEFFEAGANSNPFID-DEEEAVTF 476
  
```

The segment of gill1304599 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 131. Based on the structural similarity these homologous

25 polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the

30 amino acid sequence set out in the sequence listing as SEQ ID NO. 132 which corresponds to the "Q" sequence in the alignment shown above. Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1NIB and to a lesser extent in Soares

35 melanocyte 2NbHM; Human Testes: Soares fetal liver spleen 1NFLS; Stratagene fetal spleen (#937205); Soares\_fetal\_heart\_NbHH19W; Human Testes Tumor; Human Skin Tumor; Stratagene NT2 neuronal precursor 937230; Human Fetal Heart; Spleen. Chronic lymphocytic leukemia; Primary Dendritic Cells, lib 1; Human Fetal Spleen;



- Jurkat T-cell G1 phase; Soares\_pregnant\_uterus\_NbHPU; Human Adipose; Soares adult brain N2b5HB55Y; Human adult testis, large inserts; Human Gall Bladder; Soares\_placenta\_8to9weeks\_2NbHP8to9W; Soares\_NhHMPu\_S1; Stratagene NT2 neuronal precursor 937230; T cell helper II; Stratagene colon (#937204); Soares
- 5 ovary tumor NbHOT; CD34+ cell, I; CD34+cells, II, FRACTION 2; Human Adult Liver, subtracted; Human (HCC) cell line liver (mouse) metastasis, remake; Human Primary Breast Cancer, re-excision; Human Neutrophils, Activated, re-excision; Human T-cell lymphoma, re-excision; Human Soleus; Human Adult Heart, re-excision; B Cell lymphoma; Human Colon Cancer, re-excision; Synovial hypoxia; H.
- 10 Meningioma, M1; Human Neutrophil; Apoptotic T-cell; human ovarian cancer; Human Uterine Cancer; Human Pancreas Tumor; Human Heart; Human umbilical vein endothelial cells, IL-4 induced; Stratagene colon (#937204); Spinal cord; Soares\_fetal\_liver\_spleen\_INFLS\_S1; Epithelial-TNF $\alpha$  and INF induced; Human T-Cell Lymphoma; Soares\_NhHMPu\_S1; Stratagene neuroepithelium (#937231);
- 15 Human Placenta; Bone marrow; Human Adult Pulmonary, re-excision; Endothelial-induced; Human Amygdala; HUMAN B CELL LYMPHOMA; Human Bone Marrow, treated; Bone Marrow Cell Line (RS4,11); Human 8 Week Whole Embryo and Stratagene pancreas (#937208).

Preferred epitopes include those comprising a sequence shown in SEQ ID NO.

- 20 76 as residues: Arg-23 to Leu-28. Polynucleotides encoding such polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:31 and may have been publicly available prior to conception of
- 25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1866 of SEQ ID NO:31, b
- 30 is an integer of 15 to 1880, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 22

5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Stratagene hNT neuron (#937233); and neutrophils control.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:32 and may have been publicly available prior to conception of  
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1029 of SEQ ID NO:32, b  
15 is an integer of 15 to 1043, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 23

20 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Ovary; and Macrophage-oxLDL, re-excision.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 78 as residues: Arg-16 to Lys-21. Polynucleotides encoding such polypeptides are also provided.

25 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:33 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence  
30 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

general formula of a-b, where a is any integer between 1 to 1192 of SEQ ID NO:33, b is an integer of 15 to 1206, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.

5

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 24

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares\_testis\_NHT and to a lesser extent in Soares melanocyte 2NbHM; Human Primary Breast Cancer Reexcision; Stratagene NT2  
10 neuronal precursor 937230; Human OB HOS treated (10 nM E2) fraction I; Glioblastoma; Human Fetal Kidney; Soares breast 2NbHBst; Early Stage Human Brain; Soares\_fetal\_heart\_NbHH19W; Normalized infant brain, Bento Soares; Soares\_testis\_NHT; Human Fetal Kidney, Reexcision; Primary Dendritic cells.frac 2; Human Amygdala and Soares infant brain 1NIB.

15 Therefore, nucleic acids of the invention are useful as reagents for differential identification of the tissue(s) or cell type(s) present in a biological sample and for diagnosis of the following diseases and conditions: [insert disease condition]. Similarly, polypeptides and antibodies directed to those polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell  
20 type(s). For a number of disorders of the above tissues or cells, particularly of the [insert system where a related disease state is likely, e.g., immune], expression of this gene at significantly higher or lower levels may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to the  
25 standard gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 79 as residues: Pro-34 to Pro-40. Polynucleotides encoding such polypeptides are also provided.

30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:34 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1490 of SEQ ID NO:34, b is an integer of 15 to 1504, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 25

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Colon Tumor II and to a lesser extent in Colon Normal III; Human Eosinophils; Normal colon; Colon Carcinoma; Saos2 Cells, Untreated; Colon Tumor; Dendritic Cells From CD34 Cells; H. Meningioma, M1; Human Umbilical Vein Endothelial Cells, uninduced; Weizmann Olfactory Epithelium; Colon Normal II; Soares infant brain INIB; CD34+ cell, I, frac II; TF-1 Cell Line GM-CSF Treated; Pancreas Islet Cell Tumor; Soares\_fetal\_liver\_spleen\_INFLS\_S1; Human Adipose; Soares fetal liver spleen INFLS; Soares ovary tumor NbHOT; Thyroid Tumour; Morton Fetal Cochlea; H Female Bladder, Adult; Pancreas normal PCA4 No; Spleen metastatic melanoma; Merkel Cells; Human umbilical vein endothelial cells, IL-4 induced; Endothelial-induced; Spleen, Chronic lymphocytic leukemia; FGF enriched mixed library; Primary Dendritic cells,CapFinder2, frac 1; Prostate BPH.Lib 2, subtracted; Larynx Carcinoma; Larynx tumor; Colon Tumor; Bone marrow stroma,treated; Larynx Tumor; Human Infant Adrenal Gland; Testis, normal; Human Normal Cartilage,Fraction I; Human Normal Cartilage Fraction II; Human osteoarthritis,fraction I; Thymus; Ku 812F Basophils Line; Saos2. Dexamethosome Treated; Activated T-cells; Human Thyroid; Salivary Gland; NTERA2 teratocarcinoma cell line+retinoic acid (14 days); Human Ovary; Soares\_fetal\_heart\_NbHH19W; T-Cell PHA 16 hrs; Soares\_fetal\_liver\_spleen\_INFLS\_S1; T-Cell PHA 24 hrs; Stratagene endothelial

cell 937223; Synovial Fibroblasts (control); Soares adult brain N2b5HB55Y; Resting T-Cell Library,II; Soares\_fetal\_heart\_NbHH19W; Soares\_placenta\_8to9weeks\_2NbHP8to9W; NCI\_CGAP\_Kid3; Human Fetal Lung III; Human Testes, Reexcision and Endothelial cells-control.

- 5 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 80 as residues: Met-1 to Thr-6. Polynucleotides encoding such polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:35 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 759 of SEQ ID NO:35, b is an integer of 15 to 773, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.

## 20 FEATURES OF PROTEIN ENCODED BY GENE NO: 26

- The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil1389766 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "unknown [Homo sapiens]." A partial alignment demonstrating the observed homology is shown immediately below.

- 30 >gil1389766 unknown [Homo sapiens] >sp|Q13629|Q13629 HYPOTHETICAL 13.3 KD  
PROTEIN.  
Length = 118  
Minus Strand HSPs:

WO 00/58495

PCT/US00/07661

32

Score = 201 (70.8 bits), Expect = 5.2e-15, P = 5.2e-15  
 Identities = 47/77 (61%), Positives = 53/77 (68%), Frame = -3

```

5  O: 708 FDELGYDGSRL*SQYFGRPRADHLRSGVRDQFGQGEV-RLY*KYKNSWVWHAUVIAA 532
    *** G  GSRL +FGRPRAD+LR GV DQ GORGE L K SW WW PVI A
    S: 45 NNQAGRCGSRL--HFGRPRADYLRIGVPDORGORGESPSLLKNTKISWAWN-VPVIPA 100

    Q: 531 TQEAQAQESLEPGRQR*Q 478
    +E EA ESLEPGRQR Q
10  S: 101 IREGEAGESLEPGRQRLQ 118
  
```

The segment of gil1389766 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 143 Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art. some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 144 which corresponds to the Q sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in Human Synovial Sarcoma.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 81 as residues: Gln-40 to Ser-52. Polynucleotides encoding such polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:36 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1287 of SEQ ID NO:36. b is an integer of 15 to 1301, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 27

5 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: PERM TF274.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:37 and may have been publicly available prior to conception of  
10 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1073 of SEQ ID NO:37, b  
15 is an integer of 15 to 1087, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 28

20 It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: PERM TF274.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:38 and may have been publicly available prior to conception of  
25 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2253 of SEQ ID NO:38, b  
30 is an integer of 15 to 2267, where both a and b correspond to the positions of

nucleotide residues shown in SEQ ID NO.38, and where b is greater than or equal to a + 14.

# FEATURES OF PROTEIN ENCODED BY GENE NO: 29

5       The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil3170653 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "peroxisome assembly protein PEX10 [Homo sapiens]." A partial alignment demonstrating the observed homology is shown immediately below.

```

15      >gi|3170653 (AF060502) peroxisome assembly protein PEX10 (Homo sapiens)
      >sp|O60683|PEXA_HUMAN PEROXISOME ASSEMBLY PROTEIN PEX10
      (PEROXIN-10).
      Length = 326

      Minus Strand HSPs:

20      Score = 331 (116.5 bits), Expect = 1.4e-28, P = 1.4e-28
      Identities = 55/55 (100%), Positives = 55/55 (100%), Frame = -3

      Q: 1943 LCTLCLEERRHPTATPCGHLFCWECITAWCSSKAECPLCREKFPQKLIYLRHYR 1779
      LCTLCLEERRHPTATPCGHLFCWECITAWCSSKAECPLCREKFPQKLIYLRHYR
25      S: 272 LCTLCLEERRHPTATPCGHLFCWECITAWCSSKAECPLCREKFPQKLIYLRHYR 326
    
```

The segment of gil3170653 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 147 Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 148 which corresponds to the Q sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.



- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares infant brain 1N1B and to a lesser extent in Soares\_fetal\_heart\_NbHH19W; Human adult testis, large inserts; Human Placenta; Human Soleus; Human endometrial stromal cells-treated with estradiol;
- 5 Soares\_total\_fetus\_Nb2HF8\_9w; HL-60, PMA 4H, re-excision; Human Uterine Cancer; Stratagene pancreas (#937208); Bone Marrow Stromal Cell, untreated; Soares\_pregnant\_uterus\_NbHPU; Human Whole Six Week Old Embryo; Early Stage Human Brain; Soares melanocyte 2NbHM; Human Synovial Sarcoma; Human Fetal Lung III; Endothelial-induced; Human Cerebellum and Soares placenta Nb2HP.
- 10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:39 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1946 of SEQ ID NO:39, b is an integer of 15 to 1960, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a
- 20 + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 30

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: NCL\_CGAP\_GCB1 and to a lesser extent in
- 25 Soares\_multiple\_sclerosis\_2NbHMSP; NCL\_CGAP\_Pr22; NTERA2, control; T cell helper II; Human Epididymus; Salivary Gland, Lib 2; Soares\_multiple\_sclerosis\_2NbHMSP; Ovarian Tumor 10-3-95; Soares\_testis\_NHT; Mo7e Cell Line GM-CSF treated (1ng/ml); L428; Stromal cell TF274; Ulcerative Colitis; Soares\_pregnant\_uterus\_NbHPU; Stratagene ovarian cancer (#937219); Bone
- 30 Marrow Stromal Cell, untreated; Human adult (K.Okubo); Human fetal heart, Lambda ZAP Express; Human Gall Bladder; Human Placenta; Human Testes Tumor;

12 Week Early Stage Human II. Reexcision; Human Osteoclastoma; Bone Marrow Cell Line (RS4,11); Osteoblasts: Soares\_NhHMPu\_S1 and Nine Week Old Early Stage Human.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:40 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2006 of SEQ ID NO:40, b is an integer of 15 to 2020, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.

# FEATURES OF PROTEIN ENCODED BY GENE NO: 31

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gil1675226 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "ACF7 neural isoform 3 [Mus musculus]." A partial alignment demonstrating the observed homology is shown immediately below.

```
>gil1675226 ACF7 neural isoform 3 [Mus musculus] >sp|P97396|P97396
ACTIN-CROSSLINKING PROTEIN 7, ISOFORM 3 (ACF7 NEURAL ISOFORM
3)
      (ACF7-3) (FRAGMENT),
      Length = 2033

      Plus Strand HSPs:

      Score = 234 (82.4 bits), Expect = 2.4e-31, Sum P(2) = 2.4e-31
      Identities = 46/59 (77%), Positives = 52/59 (88%), Frame = -1

      Q: 1312 LKE-Y-LFQVWIEFGRIKLPQGYHPNDVEEHWKLIIEMLEREKSLRPAVERHWQILRV 1482
      +KE Y L +VMIEFGRIKLPQGYHPN VEEHWKLI+EMLEREKSRLPAVER +L++
      S: 525 IKELYKLEWIEFGRIKLPQGYHPNHVEEHWKLIIVMLEREKSLRPAVERLELLQI 583
```

The segment of gill1675226 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 151. Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 152 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Old Ovary; Human Whole Brain, re-excision; and Anergic T-cell.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1542 of SEQ ID NO:41, b is an integer of 15 to 1556, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 32

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Testes and to a lesser extent in Soares placenta Nb2HP; Human Kidney Medulla - unamplified; Soares\_testis\_NHT; Human

- Placenta: Soares fetal liver spleen INFLS; Human Thymus; Morton Fetal Cochlea; Soares\_placenta\_8to9weeks\_2NbHP8to9W; Human Umbilical Vein Endothelial Cells, uninduced; Stromal cell TF274; Stratagene ovarian cancer (#937219); Hemangiopericytoma: Human Fetal Brain; Human Whole Six Week Old Embryo;
- 5 Human T-Cell Lymphoma; T Cell helper I and Soares infant brain INIB.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 87 as residues: Leu-20 to Gly-26. Polynucleotides encoding such polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly
- 10 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:42 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention
- 15 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1929 of SEQ ID NO:42, b is an integer of 15 to 1943, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.

20

## FEATURES OF PROTEIN ENCODED BY GENE NO: 33

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares melanocyte 2NbHM and to a lesser extent in Spinal
- 25 cord; Breast Cancer cell line, MDA 36; 12 Week Old Early Stage Human, II; Soares\_NhHMPu\_S1; Soares\_fetal\_heart\_NbHHI9W and Human Testes.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 88 as residues: Arg- 14 to Gly-21. Polynucleotides encoding such polypeptides are also provided.

- 30 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are

related to SEQ ID NO:43 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

5 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1498 of SEQ ID NO:43, b is an integer of 15 to 1512, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.

10

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 34**

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: H. Epididymus, cauda; Human Pituitary, subt IX; Human Uterine Cancer; Spleen, Chronic lymphocytic leukemia; Soares fetal liver spleen

15 INFLS.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 89 as residues: Arg-45 to Ser-50. Polynucleotides encoding such polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly

20 available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:44 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention

25 are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1540 of SEQ ID NO:44, b is an integer of 15 to 1554, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.

30

#### **FEATURES OF PROTEIN ENCODED BY GENE NO: 35**

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares placenta Nb2HP and to a lesser extent in Soares\_pregnant\_uterus\_NbHPU; Endothelial-induced; Stratagene lung carcinoma 937218; Stratagene ovarian cancer (#937219); Human Microvascular Endothelial
- 5 Cells. fract. A; Soares infant brain 1NIB; Human Adult Liver, subtracted; Human Gall Bladder, fraction II; Human colon carcinoma (HCC) cell line, remake; Human Pancreatic Carcinoma; Human Thyroid; Human Lung; Human Prostate Cancer, Stage C fraction; Human Infant Brain; H. Kidney Medulla, re-excision; Stromal cell TF274; Human T-Cell Lymphoma; Soares melanocyte 2NbHM;
- 10 Soares\_multiple\_sclerosis\_2NbHMSP; Stratagene neuroepithelium (#937231) and Soares fetal liver spleen 1NFLS.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 90 as residues: Lys-53 to Lys-58. Polynucleotides encoding such polypeptides are also provided.

- 15 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:45 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 20 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1279 of SEQ ID NO:45, b is an integer of 15 to 1293, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a
- 25 + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 36

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in
- 30 Soares melanocyte 2NbHM; Soares placenta Nb2HP; Human Pancreas; Soares\_pineal\_gland\_N3HPG; Early Stage Human Brain; NCI\_CGAP\_GCB1; Palate

normal; Human Brain; Human Adult Liver. subtracted; Healing Abdomen  
wound, 70&90 min post incision; Resting T-Cell, re-excision; Human Pineal Gland;  
Stratagene NT2 neuronal precursor 937230; Myeloid Progenitor Cell Line: wilm's  
tumor: human ovarian cancer; Soares\_fetal\_lung\_NbHL19W; Human Umbilical Vein  
5 Endothelial Cells, uninduced; Human Chondrosarcoma; Human Testes Tumor;  
NCI\_CGAP\_Co3; NCI\_CGAP\_Co4; Normal colon: human tonsils; Human  
Amygdala; HUMAN B CELL LYMPHOMA; Soares\_pregnant\_uterus\_NbHPU;  
Stratagene HeLa cell s3 937216; Stratagene neuroepithelium NT2RAM1 937234;  
Human 8 Week Whole Embryo; Human Cerebellum; Human retina cDNA Tsp509I-  
10 cleaved sublibrary; NCI\_CGAP\_Co3; NCI\_CGAP\_GC4; NCI\_CGAP\_GCB1;  
NCI\_CGAP\_Kid5 and NCI\_CGAP\_Kid6.

Many polynucleotide sequences, such as EST sequences, are publicly  
available and accessible through sequence databases. Some of these sequences are  
related to SEQ ID NO:46 and may have been publicly available prior to conception of  
15 the present invention. Preferably, such related polynucleotides are specifically  
excluded from the scope of the present invention. To list every related sequence  
would be cumbersome. Accordingly, preferably excluded from the present invention  
are one or more polynucleotides comprising a nucleotide sequence described by the  
general formula of a-b, where a is any integer between 1 to 881 of SEQ ID NO:46, b  
20 is an integer of 15 to 895, where both a and b correspond to the positions of  
nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a  
+ 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 37

25 The computer algorithm BLASTX has been used to determine that the  
translation product of this gene shares sequence homology with, as a non-limiting  
example, the sequence accessible through the following database accession no.  
bbsl156631 (all information available through the recited accession number is  
incorporated herein by reference) which is described therein as  
30 "NF2=neurofibromatosis type 2 {alternatively spliced. form A4} [human, colorectal

cancer. Peptide Partial Mutant, 46 aa] [Homo sapiens]." A partial alignment demonstrating the observed homology is shown immediately below.

```

5      >bbs|156631 NF2=neurofibromatosis type 2 (alternatively spliced, form A4)
      [Homo sapiens] >pir|I54375|I54375 gene NF2 protein - human
      (fragment)      >sp|Q16230|Q16230 NEUROFIBROMATOSIS 2 (NF2) (FRAGMENT).
10     >gi|463121      NF2 [Homo sapiens] (SUB 8-46)
      Length = 46

      Minus Strand HSPs:
15     Score = 158 (55.6 bits), Expect = 5.1e-10, P = 5.1e-10
      Identities = 29/39 (74%), Positives = 30/39 (76%), Frame = -2

20     Q: 1539 ESCSVARAGVQVNDLGSLOPPPPRFK*FSCLGLQSSWDY 1423
      ESCSV AGVQV DLG LQP PP+FK FSCL SSWDY
      S: 7 ESCSVTLAGVQVRDLGLQLPLPKFKRFSCLSPSPSSWDY 45

```

The segment of bbs|156631 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 163. Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for determining such activities are also known in the art, some of which have been described elsewhere herein.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 164 which corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in Human Testes. Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 92 as residues: Tyr-8 to Trp-15, and Pro-42 to Lys-53. Polynucleotides encoding such polypeptides are also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:47 and may have been publicly available prior to conception of



the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the  
5 general formula of a-b, where a is any integer between 1 to 1556 of SEQ ID NO:47, b is an integer of 15 to 1570, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.

#### 10 FEATURES OF PROTEIN ENCODED BY GENE NO: 38

It has been discovered that this gene is expressed primarily in Supt cells, cyclohexamide treated, subtracted.

Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 93 as residues: Cys-80 to Thr-86. Polynucleotides encoding such polypeptides are  
15 also provided.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:48 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically  
20 excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1524 of SEQ ID NO:48, b is an integer of 15 to 1538, where both a and b correspond to the positions of  
25 nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 39

It has been discovered that this gene is expressed primarily in the following  
30 tissues/cDNA libraries: Soares fetal liver spleen 1NFLS and to a lesser extent in Soares\_fetal\_heart\_NbHH19W; Stratagene muscle 937209; Stratagene endothelial

- cell 937223; Soares breast 3NbHBst; Bone marrow; Human rejected kidney; Human Whole 6 Week Old Embryo (II), sub: Human Tonsil, Lib 3; Human Gall Bladder, fraction II; H. Atrophic Endometrium; Human Soleus; B Cell lymphoma; STROMAL-OSTEOCLASTOMA; human corpus colosum; Synovial hypoxia-RSF subtracted; H.
- 5 Kidney Cortex, subtracted; Glioblastoma; Ovarian Tumor 10-3-95; Human Thymus; Human Uterine Cancer; Stromal cell TF274; Human Hypothalamus, Schizophrenia; Human Hippocampus; Epithelial-TNF $\alpha$  and INF induced; Bone Marrow Stromal Cell, untreated; Soares\_pregnant\_uterus\_NbHPU; Stratagene colon (#937204); Stratagene hNT neuron (#937233); Human Liver, normal; NCI\_CGAP\_GCB1; Resting T-Cell
- 10 Library, II; Human T-Cell Lymphoma; Human Testes Tumor; Human Synovial Sarcoma; Activated T-Cell (12hs)/Thiouridine labelled Eco; Human Amygdala; Human Endometrial Tumor and Soares\_multiple\_sclerosis\_2NbHMSF.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:49 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the

20 general formula of a-b, where a is any integer between 1 to 1665 of SEQ ID NO:49, b is an integer of 15 to 1679, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.

## 25 FEATURES OF PROTEIN ENCODED BY GENE NO: 40

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi11710216 (all information available through the recited accession number is

30 incorporated herein by reference) which is described therein as "unknown [Homo

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sapiens]." A partial alignment demonstrating the observed homology is shown immediately below.

```

5  >gil1710216 unknown [Homo sapiens] >sp|Q99770|Q99770 HYPOTHETICAL 15.4 KD
    PROTEIN.
    Length = 139

    Plus Strand HSPs:

10  Score = 150 (52.8 bits), Expect = 1.3e-14, Sum P(3) = 1.3e-14
    Identities = 40/87 (45%), Positives = 48/87 (55%), Frame = +2

    Q:  665 YFHVSTFFFGEPRLALLPRLECSGVIFAHCNLRVLVGSRESPT*AS*VAGITVACQ---- 832
        *F SF FFF E + + RLECSG I AHCNL L GS SP AS VAG C+
15  S:  43  HFLFSFLFFFETQSHSVTRLECSGTISAHCNLCPLGSSNSPASASRVAGTAGTCRRRAQL 102

    Q:  833 -----EMGFHVTDQAGLE-HIASNDP 892
        EMGF HV + GL+ +L + P
    S:  103 IFVFLAEMGFHHVGRDGLDLNLVTHPP 129
20

```

The segment of gil1710216 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 167.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 168 which  
25 corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Prostate Cancer, Stage C fraction; Neutrophils  
30 control, re-excision; Human Neutrophil, Activated; CD34 positive cells (Cord Blood); and neutrophils control.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:50 and may have been publicly available prior to conception of  
35 the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1331 of SEQ ID NO:50, b

is an integer of 15 to 1345, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.

#### 5 FEATURES OF PROTEIN ENCODED BY GENE NO: 41

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Activated T-Cell (12hs)/Thiouridine labelledEco and to a lesser extent in Human Rhabdomyosarcoma; Human 8 Week Whole Embryo and Soares fetal liver spleen INFLS.

- 10 Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:51 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence
- 15 would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1505 of SEQ ID NO:51, b is an integer of 15 to 1519, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a
- 20 + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 42

It has been discovered that this gene is expressed primarily in Cem cells cyclohexamide treated.

- 25 Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 97 as residues: Arg-31 to Met-36. Polynucleotides encoding such polypeptides are also provided.

- Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are
- 30 related to SEQ ID NO:52 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically

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excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2001 of SEQ ID NO:52, b is an integer of 15 to 2015, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.

### FEATURES OF PROTEIN ENCODED BY GENE NO: 43

The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gnl|PIDle1349204 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "T12G3.5 [Caenorhabditis elegans]"/" A partial alignment demonstrating the observed homology is shown immediately below.

```

>gnl|PIDle1349204 T12G3.5 [Caenorhabditis elegans] >sp|Q22438|Q22438
T12G3.5
      PROTEIN.
      Length = 199

      Plus Strand HSPs:

Score = 141 (49.6 bits), Expect = 8.1e-08, P = 8.1e-08
Identities = 39/118 (33%), Positives = 64/118 (54%), Frame = +1

Q:   25 VPRLLIGIRLTLPPP--KVVDNRNEKRAMFGVYDNIIGILGNFEKHPKEL-IRGPIWLRGWK 195
    +PR+  ++ + P  KV D-W+++ A FG  D I +LG+  HP +L  P WLRG+
S:   58 LPRIPDCKVPVARPAYKVRDQNSDEAARFGQNDYIDLLGDGSHVPAQLQYHTPTWLRGFP 117

Q:   196 G---NELQPCIRKRRKRVGSRMFADD--LHNLAKRIRYLY-KH-FNRHGKFR*KRKL 342
    G  NEL + I R + +++ +  H L KRI+YL +H +N+ + +R L
S:   118 GQHKANELIKLIHYRNLYDAKLQNSPKRMWELKRIKRYLMMQHNYNKQDEIGREPNL 175

```

The segment of gnl|PIDle1349204 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 171.

Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 172 which

corresponds to the "Q" sequence in the alignment shown above (gaps introduced in a sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Human Adult Retina; Soares\_pregnant\_uterus\_NbHPU; and Human Amygdala.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:53 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 922 of SEQ ID NO:53, b is an integer of 15 to 936, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 44

It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Resting T-Cell; and neutrophils control.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:54 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1863 of SEQ ID NO:54, b is an integer of 15 to 1877, where both a and b correspond to the positions of

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nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.

#### FEATURES OF PROTEIN ENCODED BY GENE NO: 45

5       The computer algorithm BLASTX has been used to determine that the translation product of this gene shares sequence homology with, as a non-limiting example, the sequence accessible through the following database accession no. gi12947318 (all information available through the recited accession number is incorporated herein by reference) which is described therein as "supervillin [Homo sapiens]." A partial alignment demonstrating the observed homology is shown  
10 immediately below.

```
15       >gi12947318 (AF051850) supervillin [Homo sapiens] >sp|O60611|O60611
          SUPERVILLIN.
          Length = 1788

          Plus Strand HSPs:

20       Score = 170 (59.8 bits), Expect = 6.9e-09, P = 6.9e-09
          Identities = 32/32 (100%), Positives = 32/32 (100%), Frame = +2

          Q:       2  EDFEFALDMTRDEYNALPAWKQVNLKKAKGLF 97
                  EDFEFALDMTRDEYNALPAWKQVNLKKAKGLF
          S: 1757 EDFEFALDMTRDEYNALPAWKQVNLKKAKGLF 1788
```

25       The segment of gi12947318 that is shown as "S" above is set out in the sequence listing as SEQ ID NO. 183. Based on the structural similarity these homologous polypeptides are expected to share at least some biological activities. Such activities are known in the art, some of which are described elsewhere herein. Assays for  
30 determining such activities are also known in the art, some of which have been described elsewhere herein.

      Preferred polypeptides of the invention comprise a polypeptide having the amino acid sequence set out in the sequence listing as SEQ ID NO. 184 which corresponds to the Q sequence in the alignment shown above (gaps introduced in a  
35 sequence by the computer are, of course, removed). Polynucleotides encoding such polypeptides are also provided.

- It has been discovered that this gene is expressed primarily in the following tissues/cDNA libraries: Soares\_pregnant\_uterus\_NbHPU and to a lesser extent in Human Infant Brain; Human Placenta; Human Fetal Heart; Human 8 Week Whole Embryo; Soares fetal liver spleen 1NFLS; Soares melanocyte 2NbHM;
- 5 Soares\_senescent\_fibroblasts\_NbHSF; Soares\_fetal\_heart\_NbHH19W; Soares infant brain 1NIB; Morton Fetal Cochlea; Soares\_total\_fetus\_Nb2HF8\_9w; Human Soleus; Human Prostate; Stratagene lung (#937210); Human Gall Bladder; Stratagene ovarian cancer (#937219); Human Placenta; Dendritic cells, pooled; Human Synovial Sarcoma; Soares\_NbHMPu\_S1; Endothelial-induced; Human Amygdala;
  - 10 Soares\_pregnant\_uterus\_NbHPU; Human Trachea Tumor; KMH2 cell line; Namalwa Cells; Soares ovary tumor NbHOT; Soares\_fetal\_lung\_NbHL19W; Crohn's Disease; Larynx carcinoma II; Palate carcinoma; Activated T-Cells, 8 hrs, subtracted; WI 38 cells; Human Fetal Brain; Human OB MG63 treated (10 nM E2) fraction I; Human (HCC) cell line liver (mouse) metastasis, remake; Human Adult Retina; HUMAN
  - 15 STOMACH; Smooth muscle-ILb induced; H. Epididymus, caput & corpus; H. cerebellum, Enzyme subtracted; Early Stage Human Lung, subtracted; HSA 172 Cells; Human Whole Brain, re-excision; Human Amygdala, re-excision; Hepatocellular Tumor; Stratagene placenta (#937225); Healing groin wound, 7.5 hours post incision; NTERA2 + retinoic acid, 14 days; LNCAP prostate cell line;
  - 20 Soares\_pregnant\_uterus\_NbHPU; Human endometrial stromal cells-treated with progesterone; Human Frontal Cortex, Schizophrenia; HL-60, PMA 4H, re-excision; Jurkat T-Cell, S phase; Human Manic Depression Tissue; Prostate BPH; H. Lymph node breast Cancer; Human Chronic Synovitis; human ovarian cancer; Human Fetal Dura Mater; Human Hippocampus; Soares\_fetal\_lung\_NbHL19W; Spinal cord;
  - 25 Soares adult brain N2b5HB55Y; Ulcerative Colitis; Stratagene colon (#937204); Human Thymus Stromal Cells; Human Adrenal Gland Tumor; Smooth muscle, serum induced, re-exc; 12 Week Old Early Stage Human; Colon Carcinoma; Soares\_multiple\_sclerosis\_2NbHMSP; Human Adult Pulmonary, re-excision; Activated T-Cell (12hs)/Thiouridine labelled Eco; Stratagene endothelial cell 937223;
  - 30 T Cell helper I; Smooth muscle, control; HUMAN B CELL LYMPHOMA; Stratagene neuroepithelium (#937231); Activated T-cell (12h)/Thiouridine re-excision; Human



Testes: Keratinocyte, Human Cerebellum: Infant brain, LLNL array of Dr. M. Soares  
INIB: Soares\_multiple\_sclerosis\_2NbHMSP and Primary Dendritic Cells, lib 1.

Many polynucleotide sequences, such as EST sequences, are publicly  
available and accessible through sequence databases. Some of these sequences are  
5 related to SEQ ID NO:55 and may have been publicly available prior to conception of  
the present invention. Preferably, such related polynucleotides are specifically  
excluded from the scope of the present invention. To list every related sequence  
would be cumbersome. Accordingly, preferably excluded from the present invention  
are one or more polynucleotides comprising a nucleotide sequence described by the  
10 general formula of a-b, where a is any integer between 1 to 918 of SEQ ID NO:55, b  
is an integer of 15 to 932, where both a and b correspond to the positions of  
nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a  
+ 14.

Table 1

Gene No.	cDNA Clone ID	ATCC Deposit NO/Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA of SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
1	HTXCU30	203918 4/8/99	Uni-ZAP XR	11	1440	98	1440		221	56	1			14
2	HTOBG07	203918 4/8/99	Uni-ZAP XR	12	1344	1	1344		1123	57	1			4
3	HSQAX94	203918 4/8/99	Uni-ZAP XR	13	3107	814	3107	886	886	58	1	36	37	81
4	HTOFA11	203918 4/8/99	Uni-ZAP XR	14	2597	1	2597	99	99	59	1	18	19	43
5	HFFAH01	203918 4/8/99	Lambda ZAP II	15	818	1	769	72	72	60	1	23	24	50
6	HNIH165	203918 4/8/99	Uni-ZAP XR	16	303	1	303	161	161	61	1	25	26	47

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Gene No.	cDNA Clone ID	ATCC Deposit NO./Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	Signal Peptide	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA
7	HNHCP14	203918 4/8/99	Uni-ZAP XR	17	933	1	933		55	62	1	31	32	50
8	HEAAW54	203918 4/8/99	Uni-ZAP XR	18	1104	1	1104	196	196	63	1	23	24	53
9	HTDAA78	203918 4/8/99	pSport1	19	825	1	825	151	151	64	1			20
10	HSTBG23	203918 4/8/99	Uni-ZAP XR	20	1695	1	1695	96	96	65	1	26	27	31
11	HPTT165	203918 4/8/99	Uni-ZAP XR	21	1175	1	1175	210	210	66	1	13	14	38
12	HTOEA53	203918 4/8/99	Uni-ZAP XR	22	2020	1	2020	56	56	67	1	22	23	31
13	HUVD48	203918 4/8/99	Uni-ZAP XR	23	1827	1	1827	196	196	68	1			5

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Gene No.	cDNA Clone ID	ATCC Deposit NO./Z. and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
14	HSRDM56	203918 4/8/99	Uni-ZAP XR	24	2023	1	2023	134	134	69	1	27	28	73
15	HLHDC33	203918 4/8/99	Uni-ZAP XR	25	947	1	947		63	70	1			1
16	HSJBB27	203918 4/8/99	Uni-ZAP XR	26	1195	1	1195	66	66	71	1	25	26	39
17	HOUJBG39	203918 4/8/99	Uni-ZAP XR	27	1647	1	1647	62	62	72	1			26
18	HNIHAY26	203918 4/8/99	Uni-ZAP XR	28	2099	1	2099		777	73	1	23	24	36
19	HSAXL82	203918 4/8/99	Uni-ZAP XR	29	899	1	899		164	74	1	28	29	40
20	HCE3L04	203918 4/8/99	Uni-ZAP XR	30	709	1	709	221	221	75	1	16	17	46

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Gene No.	cDNA Clone ID	ATCC Deposit NO:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
21	HTTEZ02	203918 4/8/99	Uni-ZAP XR	31	1880	1	1880	250	250	76	1	22	23	28
22	HNGFN77	203918 4/8/99	Uni-ZAP XR	32	1043	1	1043	195	195	77	1	21	22	30
23	HOVAE82	203918 4/8/99	pSport1	33	1206	1	1206	154	154	78	1	17	18	37
24	HEBGM06	203918 4/8/99	Uni-ZAP XR	34	1504	9	1504	26	26	79	1	32	33	40
25	HTWBO30	203918 4/8/99	pSport1	35	773	1	773	137	137	80	1	43	44	76
26	HSSJF26	203918 4/8/99	Uni-ZAP XR	36	1301	1	1301	177	177	81	1	32	33	71
27	HPOBL67	203918 4/8/99	Lambda ZAP II	37	1087	1	1087	234	234	82	1			4

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Gene No.	cDNA Clone ID	ATCC Deposit NO:Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of AA of Signal Pep	5' NT of First AA of SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of ORF
28	HPQCB83	203918 4/8/99	Lambda ZAP II	38	2267	1	2267	85	83	1	30	31	34
29	H6EEN71	203918 4/8/99	Uni-ZAP XR	39	1960	1	1083	1434	84	1	21	22	27
30	HHEPM64	203918 4/8/99	pCMVSPORT 3.0	40	2020	520	2020	559	85	1	20	21	34
31	HOOAC84	203918 4/8/99	pBluescript	41	1556	1	1556	84	86	1	27	28	31
32	HTHAA41	203918 4/8/99	Uni-ZAP XR	42	1943	1	1943	128	87	1	14	15	32
33	HTEBL56	203918 4/8/99	Uni-ZAP XR	43	1512	1	1512	180	88	1	15	16	21
34	HUKAD46	203918 4/8/99	Lambda ZAP II	44	1554	1	1554	200	89	1	43	44	50

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Gene No.	cDNA Clone ID	ATCC Deposit NO./Z. and Date	Vector	NT SEQ ID NO:	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT of First AA of Signal Pep	AA SEQ ID NO:	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	List of AA ORF
35	HPDDT14	203918 4/8/99	pBluescript SK-	45	1293	1	1264	173	173	90	1	28	29	58
36	HPBDF31	203918 4/8/99	pBluescript SK-	46	895	1	895	149	149	91	1	23	24	29
37	HTEDH78	203918 4/8/99	Uni-ZAP XR	47	1570	1	1570	83	83	92	1	34	35	53
38	HSUSB73	203918 4/8/99	pBluescript	48	1538	1	1538	158	158	93	1	23	24	91
39	HSKAA81	203918 4/8/99	Uni-ZAP XR	49	1679	1	1679	235	235	94	1	18	19	43
40	HPECB75	203918 4/8/99	Uni-ZAP XR	50	1345	1	1345	78	78	95	1	26	27	31
41	HRDAH04	203918 4/8/99	Uni-ZAP XR	51	1519	1	1519		147	96	1	17	18	24

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Gene No.	cDNA Clone ID	ATCC Deposit NO Z and Date	Vector	NT SEQ ID NO: X	Total NT Seq.	5' NT of Clone Seq.	3' NT of Clone Seq.	5' NT of Start Codon	5' NT AA of Signal Pep	AA SEQ ID NO: Y	First AA of Sig Pep	Last AA of Sig Pep	First AA of Secreted Portion	Last AA of AA
42	HCABW10	203918 4/8/99	Uni-ZAP XR	52	2015	1	2015	76	76	97	1	24	25	40
43	HARAZ81	203918 4/8/99	pBluescript SK-	53	936	242	936	435	435	98	1	18	19	33
44	HTWAM19	203918 4/8/99	Lambda ZAP II	54	1877	1	1877	160	160	99	1			47
45	HPRCC91	203918 4/8/99	Uni-ZAP XR	55	932	1	932		653	100	1			9



Table 1 summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table 1 and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." Some of the deposits contain multiple different clones corresponding to the same gene. "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq." refers to the total number of nucleotides in the contig identified by "Gene No." The deposited clone may contain all or most of these sequences, reflected by the nucleotide position indicated as "5' NT of Clone Seq." and the "3' NT of Clone Seq." of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon." Similarly, the nucleotide position of SEQ ID NO:X of the predicted signal sequence is identified as "5' NT of First AA of Signal Pep."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The first and last amino acid position of SEQ ID NO:Y of the predicted signal peptide is identified as "First AA of Sig Pep" and "Last AA of Sig Pep." The predicted first amino acid position of SEQ ID NO:Y of the secreted portion is identified as "Predicted First AA of Secreted Portion." Finally, the amino acid position of SEQ ID NO:Y of the last amino acid in the open reading frame is identified as "Last AA of ORF."

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be

any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID

- 5 NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to proteins containing the polypeptides and the secreted
- 10 proteins encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino

15 acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide

20 sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a human cDNA of the invention deposited with the ATCC, as set forth in Table 1. The nucleotide sequence of each deposited clone can readily

25 be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and

30 determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed  
5 sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or  
10 species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or  
15 the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides  
20 are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification,  
25 such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially  
30 purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40

(1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the secreted protein.

The present invention provides a polynucleotide comprising, or alternatively  
5 consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or a cDNA contained in ATCC deposit Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y and/or a polypeptide encoded by the cDNA contained in ATCC deposit Z. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide  
10 sequence of SEQ ID NO:Y and/or a polypeptide sequence encoded by the cDNA contained in ATCC deposit Z are also encompassed by the invention.

#### Signal Sequences

The present invention also encompasses mature forms of the polypeptide having the polypeptide sequence of SEQ ID NO:Y and/or the polypeptide sequence  
15 encoded by the cDNA in a deposited clone. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone) are also encompassed by the invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from  
20 the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage  
25 specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of  
30 McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved)

protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra*.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis of the amino acid sequences of the secreted proteins described herein by this program provided the results shown in Table 1.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:Y which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides.

and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

### Polynucleotide and Polypeptide Variants

5           The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in a deposited clone.

          The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y and/or encoded by a deposited clone.

10          "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

          The present invention is also directed to nucleic acid molecules which  
15          comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence contained in a deposited cDNA clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of  
20          SEQ ID NO:Y, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the  
25          invention, as are polypeptides encoded by these polynucleotides.

          The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, the polypeptide sequence encoded by the cDNA contained  
30          in a deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown in Table 1, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject

sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is  
5 determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment,  
10 which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a  
15 matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base  
20 subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence  
25 are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence  
30 except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other



words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy  
5 terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, an amino acid  
10 sequences shown in Table 1 (SEQ ID NO: Y) or to the amino acid sequence encoded by cDNA contained in a deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject  
sequence, also referred to as a global sequence alignment, can be determined using  
15 the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2. Mismatch  
20 Penalty=1, Joining Penalty=20. Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be  
25 made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are  
30 not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by

results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced

for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

5 Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II. Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

10 Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., *J. Biol. Chem.* 268: 2984-2988  
15 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., *J. Biotechnology* 7:199-216 (1988).)

20 Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem* 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over  
25 the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in  
30 activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50

amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

#### **Polynucleotide and Polypeptide Fragments**

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention. Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or

5 deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

10 structural or functional domains, such as fragments that comprise alpha-helix and  
alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-  
forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic  
regions, alpha amphipathic regions, beta amphipathic regions, flexible regions,  
surface-forming regions, substrate binding region, and high antigenic index regions.  
15 Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are  
specifically contemplated by the present invention. Moreover, polynucleotides  
encoding these domains are also contemplated.

Biologically active fragments are those exhibiting activity similar, but not necessarily  
20 identical, to an activity of the polypeptide of the present invention. The biological  
activity of the fragments may include an improved desired activity, or a decreased  
undesirable activity. Polynucleotides encoding these polypeptide fragments are also  
encompassed by the invention.

25 polypeptide which demonstrates a functional activity. By a polypeptide  
demonstrating a "functional activity" is meant, a polypeptide capable of displaying  
one or more known functional activities associated with a full-length (complete)  
polypeptide of invention protein. Such functional activities include, but are not  
limited to, biological activity, antigenicity [ability to bind (or compete with a  
30 polypeptide of the invention for binding) to an antibody to the polypeptide of the  
invention], immunogenicity (ability to generate antibody which binds to a polypeptide



of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

The functional activity of polypeptides of the invention, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

5       For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the invention for binding to an antibody of the polypeptide of the invention, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent  
10    assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays,  
15    protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an  
20    immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand for a polypeptide of the invention identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel  
25    chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E. et al., 1995, Microbiol. Rev. 59:94-123. In another embodiment, physiological correlates of binding of a polypeptide of the invention to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in  
30    the art may routinely be applied to measure the ability of polypeptides of the invention and fragments, variants derivatives and analogs thereof to elicit related

biological activity related to that of the polypeptide of the invention (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

5

### Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that  
10 hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X),  
15 polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having  
20 antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by  
25 any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998-4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays  
30 described herein. Immunospecific binding excludes non-specific binding but does not

necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985),

5 further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30  
10 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies,  
15 that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

20 Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any  
25 combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a  
30 carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids

have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-

polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni<sup>2+</sup>-nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., *Curr. Opin. Biotechnol.* 8:724-33 (1997); Harayama, *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo and Blasco, *Biotechniques* 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in

its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

### Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and

fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding  
5 fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino  
10 acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific,  
15 trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol.  
20 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be  
25 specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

30 Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog,

herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.



Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.

58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of

numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

- 5           The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal
- 10 antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and
- 15 potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

- Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be
- 20 produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not
- 25 limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

- Methods for producing and screening for specific antibodies using hybridoma
- 30 technology are routine and well known in the art and are discussed in detail in the Examples (e.g., Example 16). In a non-limiting example, mice can be immunized

with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage

including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal

antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison. Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entirety.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can

express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a

selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

#### Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence



encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted

within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.* 278: 457-479 (1998) for a listing of human  
5 framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such  
10 methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

15 In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., *Proc. Natl. Acad. Sci.* 81:851-855 (1984); Neuberger et al., *Nature* 312:604-608 (1984); Takeda et al., *Nature* 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used.  
20 As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain  
25 antibodies (U.S. Patent No. 4,946,778; Bird, *Science* 242:423-42 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); and Ward et al., *Nature* 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the  
30 assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., *Science* 242:1038-1041 (1988)).

# Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain

thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

- 5           A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the
- 10 invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems
- 15 infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,
- 20 BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole
- 25 recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2
- 30 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which

5 direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic

10 Acids Res. 13:3101-3109 (1985); Van Hecke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence

15 of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in

20 *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be

25 utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region

30 E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad.

Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

10 In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate  
15 cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such  
20 mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable  
25 expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker.  
30 Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media.

The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule.

- 5 Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al.,  
10 Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or apr<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt,  
15 which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217  
20 (1993); May, 1993, TIB TECH 11(5):155-215); and hyg<sup>r</sup>, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993);  
25 Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

- The expression levels of an antibody molecule can be increased by vector  
30 amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA

cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino



acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or  
 5 conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are  
 10 incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody  
 15 portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers  
 20 through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO  
 25 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide.  
 30 polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for

use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used

diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ . A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine,

thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin, anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- $\alpha$ , TNF- $\beta$ , AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents

- In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications. Pinchera et al. (eds.). pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

#### Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for

the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

#### Assays For Antibody Binding

- 5       The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, 10 gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly 15 below (but are not intended by way of limitation).

- Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% 20 Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in 25 SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding 30 immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., <sup>32</sup>P or <sup>125</sup>I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al,

15                    Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.



A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC).

- 5 Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

- 10 The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

- 15 The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered
- 20 to a human patient for therapy or prophylaxis.

- It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments
- 25 thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-3}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M,  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M,
- 30  $10^{-8}$  M,  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, and  $10^{-15}$  M.

### Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or  
5 prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

10 Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science  
15 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY  
20 (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters  
25 operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA  
30 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)). In specific

embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case  
5 the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered  
10 *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct  
15 injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see,  
20 e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for  
25 cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Kolter and Smithies. Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al.,  
30 Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., *Meth. Enzymol.* 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., *Biotherapy* 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor

cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

5 In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

15 In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

20 The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

### Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment;

this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material.

- 5 including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic



acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., 5 Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such 10 compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term 15 "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline 20 solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, 25 can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as 30 pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the

practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body

fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject

has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

5 It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99m}\text{Tc}$ . The labeled antibody or antibody fragment will then preferentially  
10 accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

15 Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5  
20 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

25 Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body  
30 scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI). Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by  
5 binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of  
10 the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of  
20 bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the  
30 solid support, such as an activated carboxyl, hydroxyl, or aldehyde group.

Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound  
5 recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

### Fusion Proteins

10 Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second  
15 protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur  
20 through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell  
25 or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and  
30 specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any



combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 120:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope

derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

5

### Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In 10 the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, 15 such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac 20 promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a 25 translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin 30 resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli.

Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture  
5 mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech,  
10 Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zco, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available  
15 from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods  
20 are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from  
25 recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for  
30 purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express the polypeptide of the present invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using  $O_2$ . This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for  $O_2$ . Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J. et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1*

regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, PHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit

(see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteine acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and  
5 addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the  
10 polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose,  
15 dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between  
20 about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or  
25 lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those  
30 skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting

pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, *Therapeutics*) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or



tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides  
5 corresponding to the amino acid sequence of SEQ ID NO: Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer  
10 containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (*e.g.*, containing polypeptides having identical or different amino acid sequences) or a homotrimer (*e.g.*, containing  
15 polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (*i.e.*, polypeptides of different proteins) in addition to  
20 the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic,  
25 ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed  
30 when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion

protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence ( e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different

proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant  
5 fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced  
10 biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be  
15 employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations  
20 proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker  
25 molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the  
30 multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely

modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally,

5 techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic  
10 engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are  
15 generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by  
20 reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by  
25 reference in its entirety).

#### Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes  
30 known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V.

- 5 McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and  
10 the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the  
15 mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected  
20 individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis  
25 of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

30 In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test

subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for

obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform



5 multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point ( $T_{sub.m}$ ) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative diseases, disorders, and/or conditions are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Germann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Germann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Germann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the

level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991) ) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQA class II HLA gene, are used in forensic biology to identify individuals. (Erich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are

amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

5        There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by  
10        organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

         In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences  
15        in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

## 20        Uses of the Polypeptides

         Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

         A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression  
25        in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known  
30        in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and

technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo  
5 imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for  
10 the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or  
15 intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled antibody or antibody fragment will then  
20 preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which  
25 involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is  
30 indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a

predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

### Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldgrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry

into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the  $\beta$ -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney,



gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are  
5 complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate  
10 intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem., 265:10189-10192 (1990), which is herein incorporated by reference), in functional  
15 form.

Cationic liposomes are readily available. For example, N[1-2,3-di(oxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA ,  
20 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092  
25 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

30 Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily

available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., *Methods of Immunology*, 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and

then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell, 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA, 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA, 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

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4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al.,  
Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein  
incorporated by reference. For example, the adenovirus vector Ad2 is useful and can  
be grown in human 293 cells. These cells contain the E1 region of adenovirus and  
5 constitutively express E1a and E1b, which complement the defective adenoviruses by  
providing the products of the genes deleted from the vector. In addition to Ad2, other  
varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present  
invention.

Preferably, the adenoviruses used in the present invention are replication  
10 deficient. Replication deficient adenoviruses require the aid of a helper virus and/or  
packaging cell line to form infectious particles. The resulting virus is capable of  
infecting cells and can express a polynucleotide of interest which is operably linked to  
a promoter, but cannot replicate in most cells. Replication deficient adenoviruses  
may be deleted in one or more of all or a portion of the following genes: E1a, E1b,  
15 E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, *ex vivo* or *in vivo*,  
using an adeno-associated virus (AAV). AAVs are naturally occurring defective  
viruses that require helper viruses to produce infectious particles (Muzyczka, Curr.  
Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that  
20 may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base  
pairs of AAV can be packaged and can integrate, but space for exogenous DNA is  
limited to about 4.5 kb. Methods for producing and using such AAVs are known in  
the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678,  
5,436,146, 5,474,935, 5,478,745, and 5,589,377.

25 For example, an appropriate AAV vector for use in the present invention will  
include all the sequences necessary for DNA replication, encapsidation, and host-cell  
integration. The polynucleotide construct containing polynucleotides of the invention  
is inserted into the AAV vector using standard cloning methods, such as those found  
in Sambrook et al., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor  
30 Press (1989). The recombinant AAV vector is then transfected into packaging cells  
which are infected with a helper virus, using any standard technique, including

lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention.

- 5 These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

25 The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can  
5 be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such  
10 that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding other angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth  
15 factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

20 Preferably, the polynucleotide encoding a polypeptide of the invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be  
25 homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct  
30 needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available



depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries.

Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA, 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a

polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly

#### 15 **Biological Activities**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

#### **Immune Activity**

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases,

disorders and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide, or agonist or antagonist of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

5 A polynucleotide or polypeptide, or agonist or antagonist of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. A polynucleotide or polypeptide, or agonist or antagonist of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem  
10 cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types of hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion  
15 deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polynucleotide or polypeptide, or agonist or antagonist of the  
20 present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide, or agonist or antagonist of the present invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor  
25 deficiencies), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide, or agonist or antagonist of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or  
30 prevention of heart attacks (infarction), strokes, or scarring.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polynucleotides or

polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or  
5 polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or  
10 diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over  
15 production of cytokines (e.g., TNF or IL-1.)

### **Hyperproliferative Disorders**

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders,  
20 including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

25 For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new  
30 immune response. Alternatively, decreasing an immune response may also be a

method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstrom's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA

construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform  
5 non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said  
10 polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing  
15 expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the  
20 normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any  
25 other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA  
30 delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference.

In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes  
5 needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell  
10 proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or  
15 disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of  
20 the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of  
25 the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a  
30 mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for



producing anti-polypeptides and anti-poly-nucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

5       A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings  
10       provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

          In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing  
15       cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

          The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or  
20       hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

          It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and  
25       therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-6}M$ ,  $10^{-6}M$ ,  $5 \times 10^{-7}M$ ,  $10^{-7}M$ ,  $5 \times 10^{-8}M$ ,  $10^{-8}M$ ,  $5 \times 10^{-9}M$ ,  $10^{-9}M$ ,  $5 \times 10^{-10}M$ ,  $10^{-10}M$ ,  $5 \times 10^{-11}M$ ,  $10^{-11}M$ ,  $5 \times 10^{-12}M$ ,  $10^{-12}M$ ,  
30        $5 \times 10^{-13}M$ ,  $10^{-13}M$ ,  $5 \times 10^{-14}M$ ,  $10^{-14}M$ ,  $5 \times 10^{-15}M$ , and  $10^{-15}M$ .

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst. 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies

directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

### **Cardiovascular Disorders**

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right

ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

- Cardiovascular diseases, disorders, and/or conditions also include heart
- 5 disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular
- 10 septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and
- 15 cardiovascular tuberculosis.

- Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus
- 20 syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

- 25 Heart valve disease include aortic valve insufficiency, aortic valve stenosis, heart murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

- 30 Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis,

pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris.

- 5 coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

- Cardiovascular diseases also include vascular diseases such as aneurysms, angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, 10 aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno- 15 occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

- Aneurysms include dissecting aneurysms, false aneurysms, infected 20 aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

- Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis 25 obliterans.

- Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, 30 Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia

(including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a *Therapeutic*, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

#### **Anti-Angiogenesis Activity**

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate.

Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound

healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides,

polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma;

5 Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

10 Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate  
15 mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or  
20 conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia  
25 (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization;  
30 telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.



For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

- 5        Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably  
10        initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy,  
15        retrolental fibroplasia and macular degeneration.

- Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic  
20        retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

- 25        Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited.  
30        Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the

pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization,

- 5 including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

- 10 Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions,
- 15 prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a mucoadhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in
- 20 corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

- Within other embodiments, the compounds described above may be injected
- 25 directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbal corneal injection to "protect" the
- 30 cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization.

In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-  
5 3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide,  
10 antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be  
15 placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood  
20 vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should  
25 be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The  
30 compound may be administered topically, via intravitreal injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uviitis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti-angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide

variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

5       The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator  
10 Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the  
15 above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl  
20 complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate.  
25 calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its  
30 hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example,

molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydropoline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST": Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthranilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolimidazole; and metalloproteinase inhibitors such as BB94.

#### **Diseases at the Cellular Level**

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma,

- osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer), autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease,
- 5 polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or
- 10 metasis of cancers, in particular those listed above.

- Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including
- 15 acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's
- 20 macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic
- 25 cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor,
- 30 cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma,



craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or  
 5 antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis,  
 10 Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct injury)  
 15 and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is  
 20 provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds.  
 Polynucleotides or polypeptides, as well as agonists or antagonists of the invention,  
 25 may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia,  
 30 malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avascular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omentopial graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation. chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or

polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

- The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly.
- Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

- Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to

prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and aveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

### Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which

result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of

5 either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the

10 nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated

15 with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6)

20 lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar

25 degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating

30 disease including, but not limited to, multiple sclerosis, human immunodeficiency

virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

- In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

- The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp.

Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron  
5 dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include,  
10 but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive  
15 spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

## 20 **Infectious Disease**

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented,  
25 and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

30 Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or

polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Bimaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papilloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to,



- include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., *Corynebacterium*, *Mycobacterium*, *Nocardia*), *Cryptococcus neoformans*, *Aspergillosis*, *Bacillaceae* (e.g., *Anthrax*, *Clostridium*), *Bacteroidaceae*, *Blastomycosis*, *Bordetella*, *Borrelia* (e.g., *Borrelia burgdorferi*), *Brucellosis*, *Candidiasis*, *Campylobacter*,  
5 *Coccidioidomycosis*, *Cryptococcosis*, *Dermatocycoses*, *E. coli* (e.g., *Enterotoxigenic E. coli* and *Enterohemorrhagic E. coli*), *Enterobacteriaceae* (*Klebsiella*, *Salmonella* (e.g., *Salmonella typhi*, and *Salmonella paratyphi*), *Serratia*, *Yersinia*), *Erysipelothrix*, *Helicobacter*, *Legionellosis*, *Leptospirosis*, *Listeria*, *Mycoplasmatales*,  
10 *Mycobacterium leprae*, *Vibrio cholerae*, *Neisseriaceae* (e.g., *Acinetobacter*, *Gonorrhea*, *Menigococcal*), *Meisseria meningitidis*, *Pasteurellaceae* Infections (e.g., *Actinobacillus*, *Heamophilus* (e.g., *Heamophilus influenza type B*), *Pasteurella*), *Pseudomonas*, *Rickettsiaceae*, *Chlamydiaceae*, *Syphilis*, *Shigella spp.*, *Staphylococcal*, *Meningiococcal*, *Pneumococcal* and *Streptococcal* (e.g.,  
15 *Streptococcus pneumoniae* and Group B *Streptococcus*). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as  
20 Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., meningitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g.,  
25 cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diphtheria, botulism, and/or  
30 meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

### Regeneration

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

### Chemotaxis

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may have chemotaxis activity. A chemotactic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils,

epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotactic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotactic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

## 20 **Binding Activity**

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable

of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, *Drosophila*, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., *Current Protocols in Immun.*, 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the

polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

5       Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and retransfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

10       As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would  
15 be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby  
20 effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., Curr. Opin. Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques  
25 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination.  
30 In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone

PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured.

- 5 Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase,  
10 ion channels or phosphoinositide hydrolysis.

- All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays  
15 can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the  
20 invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

- Also, one could identify molecules bind a polypeptide of the invention  
25 experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to  
30 polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention.



Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or  
5 alternatively consist of, any combination or all of the beta pleated sheet regions in one of the polypeptide sequences of the invention.

### **Targeted Delivery**

In another embodiment, the invention provides a method of delivering  
10 compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one  
15 embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for  
20 delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides  
25 of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell  
30 that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes

known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a  
5 non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or  
10 mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

### **Drug Screening**

Further contemplated is the use of the polypeptides of the present invention, or  
15 the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

20 This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic  
25 or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

30 Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present

invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically  
5 labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present  
10 invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and  
15 washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays  
20 in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

25

#### **Antisense And Ribozyme (Antagonists)**

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited  
30 clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered

(see, for example, O'Connor, *Neurochem.*, 56:560 (1991)). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).

Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for

- 5 example, in Okano, *Neurochem.*, 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., *Nucleic Acids Research*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary
- 10 DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide.

- 15 A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoRI site on the 5' end and a HindIII site on the 3' end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl<sub>2</sub>, 10mM dithiothreitol (DTT) and 0.2 mM ATP) and then
- 20 ligated to the EcoRI/Hind III site of the retroviral vector PMV7 (WO 91/15580).

- For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide
- 25 is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

- In one embodiment, the antisense nucleic acid of the invention is produced
- 30 intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the

invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, *Nature*, 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., *Cell*, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.*, 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., *Nature*, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex: in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3' untranslated sequences of mRNAs have been shown to be effective at

inhibiting translation of mRNAs as well. See generally, Wagner, R., *Nature*, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5' - or 3' - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA.

- 5 Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5' -, 3' - or coding region of mRNA, antisense nucleic acids should be at least six  
10 nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

- The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-  
15 stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556  
20 (1989); Lemaitre et al., *Proc. Natl. Acad. Sci.*, 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., *BioTechniques*, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, *Pharm. Res.*, 5:539-549 (1988)). To this end,  
25 the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

- The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil.  
30 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine.

- 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil,
- 5 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v),
- 10 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w. and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

- 15 In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

- 20 In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-O-methylribonucleotide (Inoue et al., Nucl. Acids Res.,
- 25 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

- Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples,
- 30 phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be

prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated  
5 region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs  
10 corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well  
15 known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA corresponding to the polynucleotides of the invention; i.e., to  
20 increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo.  
25 DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of  
30 the ribozyme to destroy endogenous messages and inhibit translation. Since



ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic  
5 cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular  
10 cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

15 The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with  
20 overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present  
25 invention.

### Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as  
30 thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide

may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue.

pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

- 5 Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine
- 10 levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

15

### **Other Preferred Embodiments**

- Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide
- 20 sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1.

- Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Clone Sequence and ending with the nucleotide at about the position of the 3'
- 25 Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

- Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the Start Codon and ending with the nucleotide at about the position of the 3'
- 30 Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the 5' Nucleotide of the First Amino Acid of the Signal Peptide and ending with the nucleotide at about the position of the 3' Nucleotide of the Clone Sequence as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table 1 for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a human cDNA clone identified by a cDNA Clone Identifier in Table 1, which DNA molecule is contained in the deposit given the

5 ATCC Deposit Number shown in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said human cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide

10 sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said human

15 cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said human cDNA clone.

A further preferred embodiment is a method for detecting in a biological

20 sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained

25 in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences

30 comprises determining the extent of nucleic acid hybridization between nucleic acid

molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence  
5 selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a  
10 sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table 1; and a nucleotide sequence encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA  
15 molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1.

Also preferred is a polypeptide, wherein said sequence of contiguous amino  
20 acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions beginning with the residue at about the position of the First Amino Acid of the Secreted Portion and ending with the residue at about the Last Amino Acid of the Open Reading Frame as set forth for SEQ ID NO:Y in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
25 at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid  
5 sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino  
10 acids is included in the amino acid sequence of a secreted portion of the secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
15 at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
20 at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence  
25 at least 95% identical to the amino acid sequence of the secreted portion of the protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is an isolated antibody which binds specifically to a  
30 polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group



consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table

5 1.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules

in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a secreted protein identified in Table 1, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y

wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

5 Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino  
10 acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table 1; and a complete amino acid sequence of a secreted protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1.

Further preferred is a method of making a recombinant vector comprising  
15 inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising  
20 culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of  
25 SEQ ID NO:Y beginning with the residue at the position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y wherein Y is an integer set forth in Table 1 and said position of the First Amino Acid of the Secreted Portion of SEQ ID NO:Y is defined in Table 1; and an amino acid sequence of a secreted portion of a protein encoded by a human cDNA clone identified by a cDNA Clone Identifier in Table 1  
30 and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to  
5 increase the level of said protein activity in said individual.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit,  
10 goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

In specific embodiments of the invention, for each "Contig ID" listed in the fourth column of Table 2, preferably excluded are one or more polynucleotides  
15 comprising, or alternatively consisting of, a nucleotide sequence referenced in the fifth column of Table 2 and described by the general formula of a-b, whereas a and b are uniquely determined for the corresponding SEQ ID NO:X referred to in column 3 of Table 2. Further specific embodiments are directed to polynucleotide sequences excluding one, two, three, four, or more of the specific polynucleotide sequences  
20 referred to in the fifth column of Table 2. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 2

Gene No.	cDNA Clone ID	NT SEQ ID NO:	Contig ID	Public Accession Numbers
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WO 00/58495

PCT/US00/07661

215

		X		
1	HTXCU30	11	839846	AA604691
3	HSQAX94	13	838800	T63353, T63677, T96927, T97034, R06604, R06613, R06660, R06669, R14723, R36481, R36482, R42442, R42442, R62535, R66089, R67689, H47786, R84588, N22975, N32390, N50451, N50507, N79367, N94584, W23791, W49640, W49607, AA232656, AA234386, AA258286, AA613263, AA887754, AA972500, AA969851, AI051650, AI094753, AI097106, AA649044, AA649043, AA406244, AA478670, AA479212, AA431110, AA703127, AA770028, AA883725, AA970514, AA972808, AI074320, AI086675
15	HLHDC33	25	779499	N47880
22	HNGFN77	32	842620	AA085043
24	HEBGM06	34	842527	R54668, R59346, H97686, N31494, N44569, W58702, AA179801, AA180543, N32284, AA412420, AA412563, AA446361, AA446543, AA758971, AI023622, T17064
29	H6EEN71	39	829201	T74716, R14904, R17704, R44067, R44483, R44067, R44483, R70146, H05924, H11478, H24144, H83454, AA029577, AA029578, AA076524, AA149520, AA425537
30	HHEPM64	40	844953	N51486, N53629, N59811, N72758, AA148559, AA165330, AA235159, AA489244, AA504283, AA689472, AA689529, AA714017, AA731441, C01997, N88816, AI025597

WO 00/58495

PCT/US00/07661

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43	HARAZ81	53	832380	AA133351
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Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

5

### **Examples**

#### **Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample**

Each cDNA clone in a cited ATCC deposit is contained in a plasmid vector.  
10 Table 1 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The table immediately below correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 1 as being  
15 isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

	<u>Vector Used to Construct Library</u>	<u>Corresponding Deposited</u>
	<u>Plasmid</u>	
	Lambda Zap	pBluescript (pBS)
20	Uni-Zap XR	pBluescript (pBS)
	Zap Express	pBK
	lafmid BA	plafmid BA
	pSport1	pSport1
	pCMVSPORT 2.0	pCMVSPORT 2.0
25	pCMVSPORT 3.0	pCMVSPORT 3.0
	pCR <sup>®</sup> 2.1	pCR <sup>®</sup> 2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap  
XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos.  
5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res.  
16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res.  
30 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are

commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey  
Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and  
pBK contains a neomycin resistance gene. Both can be transformed into *E. coli* strain  
XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+  
5 and KS-. The S and K refers to the orientation of the polylinker to the T7 and T3  
primer sequences which flank the polylinker region ("S" is for SacI and "K" is for  
KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to  
the orientation of the fl origin of replication ("ori"), such that in one orientation,  
single stranded rescue initiated from the fl ori generates sense strand DNA and in the  
10 other, antisense.

Vectors pSport1, pCMVSPORT 2.0 and pCMVSPORT 3.0, were obtained from  
Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors  
contain an ampicillin resistance gene and may be transformed into *E. coli* strain  
DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et  
15 al., *Focus* 15:59 (1993).) Vector lacmid BA (Bento Soares, Columbia University,  
NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain  
XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday  
Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be  
transformed into *E. coli* strain DH10B, available from Life Technologies. (See, for  
20 instance, Clark, J. M., *Nuc. Acids Res.* 16:9677-9686 (1988) and Mead, D. et al.,  
*Bio/Technology* 9: (1991).) Preferably, a polynucleotide of the present invention  
does not comprise the phage vector sequences identified for the particular clone in  
Table 1, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number  
25 cited in Table 1 for any given cDNA clone also may contain one or more additional  
plasmids, each comprising a cDNA clone different from that given clone. Thus,  
deposits sharing the same ATCC Deposit Number contain at least a plasmid for each  
cDNA clone identified in Table 1. Typically, each ATCC deposit sample cited in  
Table 1 comprises a mixture of approximately equal amounts (by weight) of about 50  
30 plasmid DNAs, each containing a different cDNA clone; but such a deposit sample



may include plasmids for more or less than 50 cDNA clones, up to about 500 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 1. First, a plasmid is directly  
5 isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with  $^{32}\text{P}$ - $\gamma$ -ATP using T4 polynucleotide  
10 kinase and purified according to routine methods. (E.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above.  
15 The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to  
20 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5' NT and the 3' NT of the clone defined in Table 1) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase  
25 chain reaction is carried out under routine conditions, for instance, in 25  $\mu\text{l}$  of reaction mixture with 0.5  $\mu\text{g}$  of the above cDNA template. A convenient reaction mixture is 1.5-5 mM  $\text{MgCl}_2$ , 0.01% (w/v) gelatin, 20  $\mu\text{M}$  each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation  
30 at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and

the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

**Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide**

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the cDNA sequence corresponding to SEQ ID NO:X., according to the method described in Example 1. (See also, Sambrook.)

5

**Example 3: Tissue Distribution of Polypeptide**

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 1 is labeled with P<sup>32</sup> using the rediprime™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN-100™ column (Clontech Laboratories, Inc.), according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various human tissues for mRNA expression.

15

Multiple Tissue Northern (MTN) blots containing various human tissues (H) or human immune system tissues (IM) (Clontech) are examined with the labeled probe using ExpressHyb™ hybridization solution (Clontech) according to manufacturer's protocol number PT1190-1. Following hybridization and washing, the blots are mounted and exposed to film at -70 degree C overnight, and the films developed according to standard procedures.

20

**Example 4: Chromosomal Mapping of the Polynucleotides**

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose

25

30

gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

**Example 5: Bacterial Expression of a Polypeptide**

5 A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product  
10 into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning  
15 sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which  
20 expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid  
25 culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to  
30 increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded  
5 onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH  
10 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl.  
15 Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250  
20 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements  
25 operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The  
30 origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR  
5 primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

10

**Example 6: Purification of a Polypeptide from an Inclusion Body**

The following alternative method can be used to purify a polypeptide expressed in *E. coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

15 Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution  
20 containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by  
25 centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree  
30 C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C  
5 without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive  
10 Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4  
15 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using  
20 a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant  $A_{280}$  monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the  
25 above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

**Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System**

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak *Drosophila* promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., *Virology* 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified in Table 1, is amplified using the PCR protocol described in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment



then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("GeneClean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGold™ virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell

culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200  $\mu$ l of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5  $\mu$ Ci of  $^{35}$ S-methionine and 5  $\mu$ Ci  $^{35}$ S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

#### **Example 8: Expression of a Polypeptide in Mammalian Cells**

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early

promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden),  
5 pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109),  
pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1,  
Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO)  
cells.

10 Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the  
15 encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker  
20 is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the  
25 production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the  
30 CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the

cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate  
5 restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If the naturally occurring signal sequence is used to produce the secreted protein, the vector does not need a second signal peptide.  
10 Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("GeneClean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1%  
15 agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB 101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid  
20 pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu$ g of the expression plasmid pC6 a pC4 is cotransfected with 0.5  $\mu$ g of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an  
25 enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in  
30 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest

concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### **Example 9: Protein Fusions**

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 5; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note

that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

- If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:

GGGATCCGAGCCCAAATCTTCTGACAAACTCACACATGCCACCGTGC  
10 CCAGCACCTGAATTCGAGGGTGACCGTCAGTCTTCTCTTCCCCCAAAA  
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGTT  
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG  
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAGCAGTA  
CAACAGCACGTACCGTGTGGTCAGCGTCCTACCCGTCTGCACCAGGACT  
15 GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA  
ACCCCATCGAGAAAACCATCTCCAAGCCAAAGGCGAGCCCCGAGAAC  
CACAGGTGTACACCTGCCCCCATCCCGGATGAGCTGACCAAGAACCAG  
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT  
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACAAGACCACGCCT  
20 CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTG  
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA  
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG  
GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:1)

25 **Example 10: Production of an Antibody from a Polypeptide**

- The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the secreted protein is prepared and purified to render it
- 30

substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a secreted polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-

specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). Alternatively, secreted protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

#### **Example 11: Production Of Secreted Protein For High-Throughput Screening Assays**

The following protocol produces a supernatant containing a polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 13-20.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel).

Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered



Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at  $2 \times 10^5$  cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8 or 9, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a 12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degrees C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in DMEM with 1x penstrep, or CHO-5 media (116.6 mg/L of  $\text{CaCl}_2$  (anhyd); 0.00130 mg/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ; 0.050 mg/L of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ; 0.417 mg/L of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 311.80 mg/L of KCl; 28.64 mg/L of  $\text{MgCl}_2$ ; 48.84 mg/L of  $\text{MgSO}_4$ ; 6995.50 mg/L of NaCl; 2400.0 mg/L of  $\text{NaHCO}_3$ ; 62.50 mg/L of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; 71.02 mg/L of  $\text{Na}_2\text{HPO}_4$ ; .4320 mg/L of  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ; .002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic

Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of  
 Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of  
 Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-  
 Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml  
 5 of L-Asparagine-H<sub>2</sub>O; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-  
 2HCL-H<sub>2</sub>O; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0  
 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-  
 H<sub>2</sub>O; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-  
 Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalanine; 40.0  
 10 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22  
 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tyrosine-2Na-2H<sub>2</sub>O; 99.65 mg/ml of L-  
 Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of  
 Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of  
 Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319  
 15 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; and  
 0.680 mg/L of Vitamin B<sub>12</sub>; 25 mM of HEPES Buffer; 2.39 mg/L of Na  
 Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL;  
 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of  
 Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin  
 20 complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed  
 with Oleic Acid; and 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal)  
 with 2mM glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L  
 DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for  
 endotoxin assay in 15ml polystyrene conical.

25 The transfection reaction is terminated, preferably by tag-teaming, at the end  
 of the incubation period. Person A aspirates off the transfection media, while person  
 B adds 1.5ml appropriate media to each well. Incubate at 37 degrees C for 45 or 72  
 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml  
 30 deep well plate and the remaining supernatant into a 2ml deep well. The supernatants  
 from each well can then be used in the assays described in Examples 13-20.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide directly (e.g., as a secreted protein) or by the polypeptide inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

### **Example 12: Construction of GAS Reporter Construct**

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I. cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b)

Class 2 includes IFN- $\alpha$ , IFN- $\gamma$ , and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:2)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn  
5 activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines  
10 are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

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	Ligand	tyk2	JAKs			STATs	GAS(element)s or ISRE
			Jak1	Jak2	Jak3		
	<u>IFN family</u>						
5	IFN- $\alpha$ /B	+	+	-	-	1,2,3	ISRE
	IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
	IL-10	+	?	?	-	1,3	
	<u>gp130 family</u>						
10	IL-6 (Pleiotrophic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
	IL-11(Pleiotrophic)	?	+	?	?	1,3	
	OnM(Pleiotrophic)	?	+	+	?	1,3	
	LIF(Pleiotrophic)	?	+	+	?	1,3	
	CNTF(Pleiotrophic)	-/+	+	+	?	1,3	
15	G-CSF(Pleiotrophic)	?	+	?	?	1,3	
	IL-12(Pleiotrophic)	+	-	+	+	1,3	
	<u>g-C family</u>						
	IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
20	IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
	IL-7 (lymphocytes)	-	+	-	+	5	GAS
	IL-9 (lymphocytes)	-	+	-	+	5	GAS
	IL-13 (lymphocyte)	-	+	?	?	6	GAS
	IL-15	?	+	?	+	5	GAS
25	<u>gp140 family</u>						
	IL-3 (myeloid)	-	-	+	-	5	GAS (IRF1>IFP>>Ly6)
	IL-5 (myeloid)	-	-	+	-	5	GAS
30	GM-CSF (myeloid)	-	-	+	-	5	GAS
	<u>Growth hormone family</u>						
	GH	?	-	+	-	5	
	PRL	?	+/-	+	-	1,3,5	
	EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
35	<u>Receptor Tyrosine Kinases</u>						
	EGF	?	+	+	-	1,3	GAS (IRF1)
	PDGF	?	+	+	-	1,3	
	CSF-1	?	+	+	-	1,3	GAS (not IRF1)
40							

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 13-14, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:3)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCGCCCTAACTCCGCCAGTTCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTATTTATGCAGAGGCCGAGGCCGCCCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAGCTT:3' (SEQ ID NO:5)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 13-14.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 15 and 16. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as IIELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

25

#### **Example 13: High-Throughput Screening Assay for T-cell Activity.**

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway.

30

The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate  
5 stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing  
10 concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in  
15 RPMI + 10% serum with 1% Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final  
20 concentration of  $10^7$  cells/ml. Then add 1 ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask and incubate at 37 degrees C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat-GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Gentamicin, and 1% Pen-Strep. These cells are treated with  
25 supernatants containing polypeptides of the invention and/or induced polypeptides of the invention as produced by the protocol described in Example 11.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being  
30 screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.



Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degrees C until SEAP assays are performed according to Example 17. The plates containing the remaining treated cells are placed at 4 degrees C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

#### **Example 14: High-Throughput Screening Assay Identifying Myeloid Activity**

The following protocol is used to assess myeloid activity by determining whether polypeptides of the invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 12. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 12, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and

wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing  
 5 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 1 mM MgCl<sub>2</sub>, and 675 uM CaCl<sub>2</sub>. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degrees C for 36 hr.

10 The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting 1x10<sup>8</sup> cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described  
 15 growth medium, with a final density of 5x10<sup>5</sup> cells/ml. Plate 200 ul cells per well in the 96-well plate (or 1x10<sup>5</sup> cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 11. Incubate at 37 degrees C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold  
 20 induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 17.

#### **Example 15: High-Throughput Screening Assay Identifying Neuronal Activity.**

When cells undergo differentiation and proliferation, a group of genes are  
 25 activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12  
 30 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl

phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells can be assessed.

- 5       The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO:6)  
10       5' GCGAAGCTTCGCGACTCCCGGATCCGCCTC-3' (SEQ ID NO:7)

- Using the GAS:SEAP/Neo vector produced in Example 12, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1  
15       promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

- 20       PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and  
25       resuspended with pipetting up and down for more than 15 times.

- Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 11. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418  
30       for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

5 The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

10 Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add 50 ul supernatant produced by Example 11, 37°C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 17.

#### 15 **Example 16: High-Throughput Screening Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, 20 and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

25 In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class I MHC.

30 Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 11. Activators or inhibitors of NF-KB would be useful in

treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTCCC) (SEQ ID NO:8), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTCCCGGGGACTTTCGGGGACTTTCGGGGAC  
TTTCCATCTGCCATCTCAATTAG:3' (SEQ ID NO:9)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTGGCAAAGCCTAGGC:3' (SEQ ID NO:4)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5' : CTCGAGGGGACTTCCCGGGGACTTCCGGGGACTTTCGGGACTTCCATCTGCCATCTCAATTA  
GTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCCCCCTAACTCCGCCAGTTCGCCCATTC  
TCCGCCCCATGGCTGACTAATTTTTTTTATTATGCAGAGGCCGAGGCCGCCCTCGGCCTCTGAGCTATT  
CCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID  
NO:10)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly,

the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene. after restricting pGFP-1 with SalI and NotI.

- Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 13. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 13. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

#### **Example 17: Assay for SEAP Activity**

- As a reporter molecule for the assays described in Examples 13-16, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

- Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

- Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

- Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

#### **Reaction Buffer Formulation:**

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75

14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25
24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

**Example 18: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.: catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.



**Example 19: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, the identification of novel human secreted proteins capable of activating tyrosine kinase signal transduction pathways are of interest. Therefore, the following protocol is designed to identify those novel human secreted proteins capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar

Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyné Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

- 5           To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyné plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50  $\mu$ l of the supernatant produced in Example 11, the medium was removed and 100  $\mu$ l of extraction buffer ((20 mM
- 10   HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4 degrees C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane
- 15   bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degrees C at 16,000 x g.
- 20           Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

- Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a
- 25   biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

- The tyrosine kinase reaction is set up by adding the following components in
- 30   order. First, add 10  $\mu$ l of 5  $\mu$ M Biotinylated Peptide, then 10  $\mu$ l ATP/Mg<sub>2+</sub> (5  $\mu$ M ATP/50  $\mu$ M MgCl<sub>2</sub>), then 10  $\mu$ l of 5x Assay Buffer (40  $\mu$ M imidazole hydrochloride,

pH 7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degrees C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

- 5        The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degrees C for 20 min. This allows the streptavidin coated 96 well plate to associate with the  
10        biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degrees C for one hour. Wash the well as above.

- 15        Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

20        **Example 20: High-Throughput Screening Assay Identifying Phosphorylation Activity**

- As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 19, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be  
25        used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by  
30        substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against  
5 Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degrees C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and  
10 cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 11 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As  
15 a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive  
20 incubations with Europium-streptavidin and Europium fluorecence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation.

**Example 21: Method of Determining Alterations in a Gene Corresponding to a**  
25 **Polynucleotide**

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest  
30 in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Mannheim), and FISH performed as described in Johnson et al., *Methods Cell Biol.* 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

**Example 22: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample**

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide  
5 is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with  
10 specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample  
15 containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature.  
20 The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard  
25 curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

**Example 23: Formulation**

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a

- 5     Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

- 10     The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

- 15     As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1  $\mu\text{g/kg/day}$  to 10  $\text{mg/kg/day}$  of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01  $\text{mg/kg/day}$ , and most preferably for humans between about 0.01 and 1  $\text{mg/kg/day}$  for the hormone. If given continuously, the Therapeutic is typically administered at a  
20     dose rate of about 1  $\mu\text{g/kg/hour}$  to about 50  $\mu\text{g/kg/hour}$ , either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears  
25     to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray.

- "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid  
30     filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include

intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317-327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos.



4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

5 In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

10 Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and  
15 concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both.  
20 Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

25 The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts: antioxidants such as ascorbic acid; low molecular weight (less than about ten  
30 residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as

- polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic
- 5 surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

- 10 Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

- 15 Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by
- 20 reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

- The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or
- 25 biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

- The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the
- 30 Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG.

and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to,

5 Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, haemophilus influenzae B,

10 whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic

15 mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in

20 combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either

25 concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination"

30 further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-1BB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIVIXAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and

VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

- 5 In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, 10 RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ 15 (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any 20 combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or 25 prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with 30 FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to

prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide, methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressant preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMUNE™/NEORAL™/SANGDYA™

(cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucocorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

- 5 In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™.
- 10 In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

- In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-
- 15 acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.
- 20

- In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin,
- 25
- 30 mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine,

hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g.,  
 5 mephallen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in  
 10 combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of the components of  
 15 CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered  
 20 with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are  
 25 administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as  
 30 disclosed in European Patent Number EP-282317; Placental Growth Factor (PIGF), as disclosed in International Publication Number WO 92/06194; Placental Growth



Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth  
5 Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth  
10 Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are  
15 administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are  
20 administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in  
25 combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

#### **Example 24: Method of Treating Decreased Levels of the Polypeptide**

The present invention relates to a method for treating an individual in need of  
30 an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically

effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the  
5 secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily  
10 dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 23.

#### **Example 25: Method of Treating Increased Levels of the Polypeptide**

15 The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

20 In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense  
25 polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 23.

#### **Example 26: Method of Treatment Using Gene Therapy-Ex Vivo**

30 One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a

subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24  
5 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several  
10 days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is  
15 fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site  
20 and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto  
25 agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the  
30 gene is then added to the media and the packaging cells transduced with the vector.

The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

#### **Example 27: Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides of the Invention**

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA*, 86:8932-8935 (1989); and Zijlstra et al., *Nature*, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be

operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM  $\text{Na}_2\text{HPO}_4$ , 6 mM dextrose). The cells are recentrifuged, the

supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately  $3 \times 10^6$  cells/ml. Electroporation should be performed immediately following resuspension.

- 5 Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3' end. Two non-coding sequences are amplified via PCR: one
- 10 non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3' end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5' end and a HindIII site at the 3' end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The
- 15 resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

- Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120  $\mu\text{g/ml}$ . 0.5 ml of the cell suspension (containing approximately  $1.5 \times 10^6$  cells) is then added to the cuvette,
- 20 and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960  $\mu\text{F}$  and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of
- 25 approximately 14-20 mSec should be observed.

- Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following
- 30 day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

5

**Example 28: Method of Treatment Using Gene Therapy - In Vivo**

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 10 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier. 20

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. 25 (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art. 30

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence



can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15  $\mu$ m cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

**Example 29: Transgenic Animals.**

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*,  
5 baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (*i.e.*,  
10 polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus  
15 mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (*see, e.g.*, Ulmer et al., Science  
20 259:1745 (1993); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); *etc.* For a review of such techniques, *see* Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

25 Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campbell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

30 The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their

cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more

than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given  
5 integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

10 Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

15

#### **Example 30: Knock-Out Animals.**

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., *Nature* 317:230-234 (1985); Thomas & Capecchi, *Cell* 51:503-  
20 512 (1987); Thompson et al., *Cell* 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable  
25 marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in  
30 research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas &

Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

5 In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not  
10 limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention,  
15 e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve  
20 expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the  
25 body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

30 When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which

prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

- 5 Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

10

#### **Example 31: Production of an Antibody**

##### **a) Hybridoma Technology**

- The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells  
15 expressing polypeptide(s) of the invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of polypeptide(s) of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

- 20 Monoclonal antibodies specific for polypeptide(s) of the invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized  
25 with polypeptide(s) of the invention or, more preferably, with a secreted polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml  
30 of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC. After fusion, the resulting hybridoma cells are  
 5 selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide(s) of the invention.

Alternatively, additional antibodies capable of binding to polypeptide(s) of the  
 10 invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to  
 15 produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the protein-specific antibody can be blocked by polypeptide(s) of the invention. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and are used to immunize an animal to induce formation of further protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such  
 20 antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214  
 25 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

30           b) Isolation Of Antibody Fragments Directed Against  
               Polypeptide(s) From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide(s) of the invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

5       Rescue of the Library.

A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-AMP-GLU) and  
10       grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY  
15       containing 100 µg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III  
20       particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-  
25       AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

30       Panning of the Library.



Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100  $\mu\text{g/ml}$  or 10  $\mu\text{g/ml}$  of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100  $\mu\text{g/ml}$  ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

#### Characterization of Binders.

Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10  $\mu\text{g/ml}$  of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

**Example 32: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

**In Vitro Assay:** Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive

cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay: BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 33: T Cell Proliferation Assay**

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100  $\mu\text{L}$ /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1  $\mu\text{g}/\text{mL}$  in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells ( $5 \times 10^4$ /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200  $\mu\text{L}$ ). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100  $\mu\text{L}$  of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100  $\mu\text{L}$  of medium containing 0.5  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

**Example 34: Effect of Polypeptides of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and

MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional

- 5 maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies  
10 for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-  
15 12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10<sup>6</sup>/ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for  
20 IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion  
25 molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

30 FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS

(positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

5

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

15

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

25

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with

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- increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

- Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well.
- Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of  $H_2O_2$  produced by the macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

- The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polypeptides, polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

## **Example 35: Biological Effects of Polypeptides of the Invention**

### **Astrocyte and Neuronal Assays**

- Recombinant polypeptides of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1

and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by EIA



kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1 $\alpha$  for 24 hours. The supernatants are collected and  
5 assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

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#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively  
15 characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the  
20 electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989).  
25 Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated to  
30 determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic

neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 36: The Effect of Polypeptides of the Invention on the Growth of Vascular Endothelial Cells**

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2.5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying

concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

- 5 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

10 **Example 37: Stimulatory Effect of Polypeptides of the Invention on the Proliferation of Vascular Endothelial Cells**

- For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF<sub>165</sub> or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak *et al.*, *In Vitro Cell. Dev. Biol.* 30A:512-518 (1994).

- 25 The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 38: Inhibition of PDGF-induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect**

- HAoSMC proliferation can be measured, for example, by BrdUrd incorporation.
- 5 Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with 10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and
  - 10 then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for
  - 15 individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem. 6:271(36):21985-21992 (1996).

- The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to
- 20 test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 39: Stimulation of Endothelial Migration**

- This example will be used to explore the possibility that a polypeptide of the
- 25 invention may stimulate lymphatic endothelial cell migration.

- Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980:33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours
- 30 at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25

ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber,  $2.5 \times 10^5$  cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO<sub>2</sub> to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

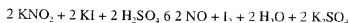
The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 40: Stimulation of Nitric Oxide Production by Endothelial Cells**

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:



The standard calibration curve is obtained by adding graded concentrations of  $\text{KNO}_3$  (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and  $\text{H}_2\text{SO}_4$ . The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per  $1 \times 10^6$  endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak *et al. Biochem. and Biophys. Res. Comm.* 217:96-105 (1995).

The studies described in this example tested activity of polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 41: Effect of Polypeptides of the Invention on Cord Formation in Angiogenesis**

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured *in vitro*.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the *in vitro* angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 µl/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 µg Cell Applications' Chord Formation Medium containing control

buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

- 5           Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-estradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

          The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to  
10   test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 42: Angiogenic Effect on Chick Chorioallantoic Membrane**

- Chick chorioallantoic membrane (CAM) is a well-established system to examine  
15   angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

          Fertilized eggs of the White Leghorn chick (*Gallus gallus*) and the Japanese quail (*Coturnix coturnix*) are incubated at 37.8°C and 80% humidity. Differentiated CAM of  
20   16-day-old chick and 13-day-old quail embryos is studied with the following methods.

          On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in  
25   distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

- 30           The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 43: Angiogenesis Assay Using a Matrigel Implant in Mouse**

5        *In vivo* angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new  
10 blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the  
15 mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after  
20 staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

The studies described in this example tested activity of a polypeptide of the  
25 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.



**Example 44: Rescue of Ischemia in Rabbit Lower Limb Model**

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshita *et al.*, *Am J. Pathol* 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen *et al.*, *Hum Gene Ther.* 4:749-758 (1993); Leclerc *et al.*, *J. Clin. Invest.* 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number in the rabbit thigh. (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

The studies described in this example tested activity of polynucleotides and polypeptides of the invention. However, one skilled in the art could easily modify the exemplified studies to test the agonists, and/or antagonists of the invention.

**Example 45: Effect of Polypeptides of the Invention on Vasodilation**

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900  
5 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as  $p < 0.05$  vs. the response to buffer alone.

The studies described in this example tested activity of a polypeptide of the  
10 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 46: Rat Ischemic Skin Flap Model**

15 The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

- 20
- a) Ischemic skin
  - b) Ischemic skin wounds
  - c) Normal wounds

The experimental protocol includes:

- 25
- a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).
  - b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).
  - c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.
  - d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for  
30 histological, immunohistochemical, and in situ studies.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

5

**Example 47: Peripheral Arterial Disease Model**

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

10

a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3

15

weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

20

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

25

**Example 48: Ischemic Myocardial Disease Model**

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

30

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyses.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

#### **Example 49: Rat Corneal Wound Healing Model**

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

- a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.
- b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.
- c) Making a pocket (its base is 1-1.5 mm from the edge of the eye).
- d) Positioning a pellet, containing 50mg- 5ug of a polypeptide of the invention, within the pocket.
- e) Treatment with a polypeptide of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

**Example 50: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models**

**A. Diabetic db+/db+ Mouse Model.**

5 To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than  
10 contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single  
15 autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am.*  
20 *J. of Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These  
25 homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh. *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

30 Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The

- animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional
- 5 Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

- Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01
- 10 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The
- 15 wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

- Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily
- 20 measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

- A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups
- 25 received 50mL of vehicle solution.

- Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

- 30 Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control. 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

### B. Steroid Impaired Rat Model

- The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

- To demonstrate that a polypeptide of the invention can accelerate the healing process. the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

- Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.



The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

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#### **Example 51: Lymphedema Animal Model**

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's

Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under

brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the  
5 limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control  
10 legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon  
15 sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

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**Example 52: Suppression of TNF alpha-induced adhesion molecule expression by a Polypeptide of the Invention**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules  
25 (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium  
30 determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The

local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF- $\alpha$ ), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF- $\alpha$  induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF- $\alpha$  treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90  $\mu$ l of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10  $\mu$ l of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution

Then add 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with

PBS+(Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^6$ )  $> 10^{0.5} > 10^{-1} > 10^{-1.5}$ . 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

WO 00/58495

PCT/US00/07661

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Applicant's or agent's file reference number	PS531PCT	International application No.	unassigned
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## INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>N/A</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depositary institution American Type Culture Collection	
Address of depositary institution (including postal code and country) 10801 University Boulevard Manassas, Virginia 20110-2209 United States of America	
Date of deposit 8 April 1999	Accession Number 203918
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet <input type="checkbox"/>	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
Europe In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28 (4) EPC).	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

For receiving Office use only
<input checked="" type="checkbox"/> This sheet was received with the international application
AUTHORIZED OFFICER <b>SIGNED AND POSTAL</b> <b>INTERNATIONAL DIVISION</b> 703 305 2500

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Authorized officer

WO 00/58495

PCT/US00/07661

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**ATCC Deposit No.:203918****CANADA**

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

**NORWAY**

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

**AUSTRALIA**

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

**FINLAND**

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

**UNITED KINGDOM**

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.



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ATCC Deposit No.: 203918

**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

**SWEDEN**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

**NETHERLANDS**

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group
- 5 consisting of:
  - (a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a
  - 10 polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a
  - 15 polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X;
  - (e) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X,
  - 20 having biological activity;
  - (f) a polynucleotide which is a variant of SEQ ID NO:X;
  - (g) a polynucleotide which is an allelic variant of SEQ ID NO:X;
  - (h) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;
  - 25 (i) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(h), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a secreted protein.
- 5 3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.
- 10 4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in ATCC Deposit No:Z, which is hybridizable to SEQ ID NO:X.
- 15 5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
- 20 6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.
- 25 7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.
8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.
9. A recombinant host cell produced by the method of claim 8.
- 30 10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:
  - (a) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence  
5 included in ATCC Deposit No:Z;
  - (b) a polypeptide fragment of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z, having biological activity;
  - (c) a polypeptide domain of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - 10 (d) a polypeptide epitope of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (e) a secreted form of SEQ ID NO:Y or the encoded sequence included in ATCC Deposit No:Z;
  - (f) a full length protein of SEQ ID NO:Y or the encoded sequence included in  
15 ATCC Deposit No:Z;
  - (g) a variant of SEQ ID NO:Y;
  - (h) an allelic variant of SEQ ID NO:Y; or
  - (i) a species homologue of the SEQ ID NO:Y.
12. The isolated polypeptide of claim 11, wherein the secreted form or the  
20 full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.
13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.  
25
14. A recombinant host cell that expresses the isolated polypeptide of claim 11.
15. A method of making an isolated polypeptide comprising:
  - 30 (a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

5 17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

10 18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

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19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

20 (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

25 (a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

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22. A method of identifying an activity in a biological assay, wherein the method comprises:

- (a) expressing SEQ ID NO:X in a cell;
- (b) isolating the supernatant;
- 5 (c) detecting an activity in a biological assay; and
- (d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

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<120> 45 Human Secreted Proteins

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<210> 15
<211> 818
<212> DNA
<213> Homo sapiens

<220>
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<222> (595)
<223> n equals a,t,g, or c

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<210> 16
<211> 303
<212> DNA
<213> Homo sapiens

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ccc						303

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 <212> DNA  
 <213> Homo sapiens

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 <222> (781)  
 <223> n equals a,t,g, or c

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gagatttggc	atgtgatggg	cagttctaca	tttattctct	tttttccctt	tcttttattt	240
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 <212> DNA  
 <213> Homo sapiens

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9

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&lt;210&gt; 21

&lt;211&gt; 1175

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

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&lt;210&gt; 22

&lt;211&gt; 2020

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 22

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&lt;210&gt; 23

&lt;211&gt; 1827

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (601)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (918)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1825)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 23

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&lt;210&gt; 24

&lt;211&gt; 2023

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (486)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 24

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12

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 <213> Homo sapiens  
  
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 <221> SITE  
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 <223> n equals a,t,g, or c

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 <211> 1195  
 <212> DNA  
 <213> Homo sapiens

<400> 26	
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tccctgcctt	agtgtctctt tgctgtttct gcctggacct ggagagatta caactatcga 180
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13

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 <211> 1647  
 <212> DNA  
 <213> Homo sapiens

<400> 27

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 <212> DNA  
 <213> Homo sapiens

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 <222> (644)  
 <223> n equals a,t,g, or c

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 <212> DNA  
 <213> Homo sapiens

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 <212> DNA  
 <213> Homo sapiens

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15

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709

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<220>
<221> SITE
<222> (655)
<223> n equals a,t,g, or c
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16

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 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (927)  
 <223> n equals a,t,g, or c

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<210> 33  
 <211> 1206  
 <212> DNA  
 <213> Homo sapiens

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[illegible]

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18

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tgattgagcc cgtgaggttg aggtctacgt gagctgtgat cgtgccactg caacttagtc      720
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<213> Homo sapiens

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tcttcactgc aaaaatagct ataacatgg taatatgaac aactactgca gtcaggatga      180
tcattgtcaa ctcacaactc tcgactggcc ctgggatgcc ctgcatgctg ctctcagtgt      240
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<210> 37
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<212> DNA
<213> Homo sapiens

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taagggaagc aaatgcagga aagctctcta ctctctctct cttgtctta aagcagaca      420

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20

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 <212> DNA  
 <213> Homo sapiens

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<220>  
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<220>  
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 <223> n equals a,t,g, or c

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&lt;210&gt; 40

&lt;211&gt; 2020

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 40

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 <212> DNA  
 <213> Homo sapiens

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<210> 42  
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 <212> DNA  
 <213> Homo sapiens

<400> 42

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&lt;210&gt; 43

&lt;211&gt; 1512

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 43

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aaaaaaaaaa	aa					1512

&lt;210&gt; 44

&lt;211&gt; 1554

&lt;212&gt; DNA

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&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (847)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (888)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 44

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&lt;211&gt; 1293

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 45

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&lt;210&gt; 46

&lt;211&gt; 895

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 46

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&lt;210&gt; 47

&lt;211&gt; 1570

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (412)

&lt;223&gt; n equals a,t,g, or c

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (502)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 47

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<210> 48  
 <211> 1538  
 <212> DNA  
 <213> Homo sapiens

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 <213> Homo sapiens



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<211> 1345

<212> DNA

<213> Homo sapiens

<400> 50

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tgaactctga	gattacagtt	ccctctctc	ctctccagct	gcgcctatga	accagcgggc	360
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ccctcgtcaa	gatcaaaagg	actcaaaagc	agccccagca	ctccaggacca	tttgtgctca	480
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ggcagcttgc	ctcatattgg	tcgtaaaaaa	aggacttttt	tttatctctg	ccaataaac	600
gcaaaaacta	aaaagggaag	ccgttgtata	ggaaagaatt	acatcttctc	aagtgttttt	660
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caggcttgag	tgcagttgtt	tgatcttcgc	tcacttgcaa	ctccgcctcg	tcggtctaa	780
ggagctctct	atctcaaacg	ctcagtagtc	tgggtataga	ctgcctcgcc	aagagatggg	840
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aagtagttct caagttctag agacttggag aatccaggaa tcaacaatgt cgtggaactc 1320  
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<210> 51
<211> 1519
<212> DNA
<213> Homo sapiens
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[illegible]

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<210> 52
<211> 2015
<212> DNA
<213> Homo sapiens
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[illegible]

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ttgtccatta	ttttatttta	cttttaaaat	ttattttata	ttatactttt	gtcaatgtag	1020
tgagtgtaaa	gtaatatctc	attatgtgtt	tagctcagaa	gtacaactgc	cggatgtcag	1080
gataatacaa	atagttttcc	aaggcttcct	ttaccacctc	ctgctcctgt	atctaaatttc	1140
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accagtgagg	agctttttcat	gtattattta	gctatttcag	tttcccttca	tgtagaataa	1260
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acatgagctc	tgataccctc	tgtaaaatat	gccccattaa	tgtgcaatga	caactgtgtc	1680
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taatatatac	aatgggcaag	tcacactact	tgttcatcag	gaaattctaa	ctattttctc	1860
tttatcttcc	atatgaatat	tggaaatgag	tcactaaatt	ccatgcctac	attgtgactg	1920
gaattacatt	gagactaaag	atcaatttgg	gaagaaatgc	catccttata	ataatataac	1980
aataaatcct	taattattaa	aaaaaaaaaa	aaaaa			2015

&lt;210&gt; 53

&lt;211&gt; 936

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (699)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 53

gcggtgtgcc	acagctacct	gggtgtgcct	agattgatcg	gtataaggct	cactctcccg	60
ccccccaaag	tggttgtatc	ttggaacgag	aaaagggcc	tgttcggagt	gtatgacaac	120
atcgggagtc	tgggaaactt	tgaaaagcac	cccaaagaac	tgatcagggg	gcccatatgg	180
cttcgagggt	ggaaagggaa	tgaattgcac	cgttgtatcc	gaaaggagg	aatggttgga	240
agtagaattg	tgcgtgatga	cctgcacac	cttaataaac	gcacccgcta	tctctacaaa	300
cactcttaacc	gacatgggaa	gtttcgatag	aagagaaaag	tgagaaactc	ggaaaaggct	360
catctgtcac	cctggagaag	ggaaactgta	cttttccctg	tgaggaaaag	gctttgtatt	420
ttctctgttaa	taaaaatggg	cttcttttga	atttgtatgc	cacatacttt	tgttttctct	480
tagatgcgcg	ctctactcat	gtagactatg	aagctcttag	tcactcctga	tctttaggatc	540
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aagaaaagt	ggtgagaatg	ttatttggcg	tttttgacat	aatgcgtcta	tgtymatagt	660
gtttcttaag	agcctctccac	actgaaatga	aatataagna	gaaaaatgga	gtcactactc	720
tgtactactc	tcaaaaaata	tttattaggc	atatctctct	agtaggaaac	ataggaaaaa	780
caaaaaagtg	tgaggtgtcc	tttccattta	aatatgaaac	attaggtacc	atatgaatgg	840
tagagatggc	ggatactaa	cctgaactgt	catcagtttt	ctgggtgttt	taacatttca	900
aaagcctagt	cctaaccctc	aaagattgat	cagtag			936

&lt;210&gt; 54

&lt;211&gt; 1877

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (854)

&lt;223&gt; n equals a,t,g, or c

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&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (1422)

&lt;223&gt; n equals a,t,g, or c

&lt;400&gt; 54

gaattcggca	cgagctgaat	tccatgtttc	attactgggt	agaagtaca	gaacaaatgg	60
atttagctta	ttataaggag	aagttttcta	attgttagaa	tttatcatc	tgtggaatga	120
ggctatttgt	caactagag	agttcttttt	cccttaaa	tgtagtaga	gagcagtgcg	180
aacatctcac	aagggatggt	agatattctt	ctgtggatag	atcttgaca	tgatgacctc	240
ctgggtcttc	ccagccctgt	gattctgaga	tcacatgccc	atccaggaa	ctcactgttc	300
tagaccctac	cactaaaatg	aatcatcatc	ttattccat	ctaaagcccc	ttacmagttt	360
attagtatca	tccttttcag	tggaatgcg	aaatatttta	ttcagttatg	ctaattgta	420
tagctcataa	tgtaaaatgc	ttgagaagtt	gaggttgtag	ttctgctggg	tattgtttc	480
tactgtcaaa	tatcatacca	tcagtgaat	tggtcataac	caccaaaatt	aaacattagc	540
cccttcagcc	ctctttccac	ctttatcata	tttatttgct	taacaaaatt	atcttttttt	600
tttttttact	cattgattct	gaatctccct	ctattttaa	gtatattctg	tgacagcagg	660
ctctcaacct	tggtgacca	aggaccatt	ttgtggaa	cagttttccc	accgacaggg	720
cgacgaacag	ggatggtttt	gggatgaa	cgtttccact	cagatcagat	catcaggcat	780
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cgctcttcta	tganaatcta	atgccaccac	tgactctgac	ggargcggag	ctcaggttgt	900
amtgcgaagc	gtggggagtg	gctctgaata	cagatgaagc	tttgctcatt	caactgccac	960
tcacctactg	ctatgtagcc	cagttccctaa	caggcttccg	aatgggggat	gggaacctcg	1020
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tggtaacatt	ttctgtaagg	gaatccgtgt	cttttgttgg	ctttttaa	ggccacctaa	1140
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ttgaattctg	gaaggataat	tgcatctcta	atattttgtc	ttcccttcca	aaatcatgct	1260
agttagtatt	ttcttcttta	ttcaagattt	cttttatgcc	aaatagtaaa	ttttggtaat	1320
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aatctctatt	ttttaagttc	tttaagcata	tagataatta	agaaaataaa	ttggctaggc	1560
actgtggctc	atgcctgtaa	tcccatcact	ttggggagct	gaagtgggtg	gattgcctga	1620
ggtcaggatt	tcaagaccag	cttgaccaat	atggggaaac	cccatctcta	ctaaaaatag	1680
aaaagttagc	tgggcatggg	ggcatgcgcc	tgtagtccca	gctattcagg	aggctgagac	1740
aggagaattg	cttgaaccgg	gtagaattgc	ttgaaccggg	gaggcggagg	ttgcagtgag	1800
cgagatcat	gccattgcac	tccagttcag	gcaacagggc	aaaactccat	ctccaaaaaa	1860
aaaaaaaa	aactcga					1877

&lt;210&gt; 55

&lt;211&gt; 932

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 55

cgaaagactc	gagtttgac	tagacatgac	gagggatgaa	tacaacgcc	tgcccgcctg	60
gaagcaggtg	aactggaaga	aagcaaaagg	ctgtttctga	gtggggagac	gcagagggag	120
ctccacggtc	agctccaaca	acaccactgc	accagggaaa	tgtagatata	tttttggaat	180
ggtgtttctc	acaaagtatg	tttcaatcag	agttttcaga	acctgacatt	gttaaaagata	240
ctcgtgtccc	cggagttgtg	tattttgtaa	atgtttcaagg	gaactgtttg	gaaacttctc	300
tgccacattt	caggaggttg	tcagaattaa	taaaagatc	tgtagttgac	acttaagccg	360
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tttgaagcag	ttctcttgtt	aaagtgttat	tttgatagtt	tggtgattct	aaaatatata	480
ttattttata	taaacaccat	ataagtcaaa	tatgtattta	acaaagcaat	atgtatctat	540
tcactttcaa	gatttgttgt	ggtgtcaaaa	taacatgaaa	aggttagatc	agtgctctct	600
gttgaaatag	ctctgccacc	aataatgata	ttcatacacg	tttggaaact	tttctgcagc	660
cattaggtat	gacttggtct	gagtaactgt	tccgggtgcta	aaatgaacaa	agaaatttgta	720

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cttaaatggca	tggactctgg	agaatctatg	cgaatcaacc	tttctacctt	aatatctccc	780
caaaaaatgta	tagtgcccttg	tttttatgta	cagtttatat	acagaaaagt	ttgctctgca	840
tttttgatga	tggtttgga	cattatctac	aattttactc	tcaaatagtc	aaaataaaaa	900
catctcaatt	tctaaaaaaa	aaaaaaaaaa	aa			932

<210> 56  
 <211> 15  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (15)  
 <223> Xaa equals stop translation

<400> 56  
 Ala Ala Leu Trp Gln Ser Leu Leu Phe Cys Phe Val Cys Phe Xaa  
           1                  5                  10                  15

<210> 57  
 <211> 5  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (5)  
 <223> Xaa equals stop translation

<400> 57  
 Cys Thr Leu Leu Xaa  
           1                  5

<210> 58  
 <211> 82  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (22)  
 <223> Xaa equals any of the naturally occurring L-amino acids

<220>  
 <221> SITE  
 <222> (82)  
 <223> Xaa equals stop translation

<400> 58  
 Met Glu Cys Leu Leu Tyr Arg Thr Ile Cys Leu Phe Tyr Phe Leu Val  
           1                  5                  10                  15

Arg Phe Leu Phe Ser Xaa Leu Leu Leu Met Lys Leu Phe Leu Pro Ile  
           20                  25                  30

Ile Asn Pro Asn Ser Trp Thr Leu Trp Leu Gly Asn Ser Leu Asn Phe

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35 40 45  
Gln Pro Tyr Gly Ile Ile Val Ser Leu Ser Leu Ser Leu Leu Ser  
50 55 60  
Leu Phe Ser Ser Ser Pro Tyr Phe Leu Ser Asn Lys Tyr Cys Leu Leu  
65 70 75 80  
Ile Xaa

<210> 59  
<211> 44  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (44)  
<223> Xaa equals stop translation

<400> 59  
Met Leu Leu Pro Ile Leu Leu Tyr Ser Phe Leu Tyr Pro Thr Ile Val  
1 5 10 15  
Ser Arg Asn Tyr Phe Lys Leu Ile Arg Ser Cys Met Leu Phe Leu Lys  
20 25 30  
Lys Lys Lys Lys Lys Glu Arg Lys Ile Ser Val Xaa  
35 40

<210> 60  
<211> 51  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (51)  
<223> Xaa equals stop translation

<400> 60  
Met Ala Gln Ile Thr Trp Ile His Leu Ser Val Val Ile Cys Ser  
1 5 10 15  
Ala Pro Pro Arg Arg Leu His Arg Gln His Ser Ala Val Gln Ala Trp  
20 25 30  
Ala Ala His Arg Glu His Val Gln Pro Ser Leu Thr Ser Ser Gly Arg  
35 40 45  
Met Pro Xaa  
50

<210> 61  
<211> 47

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&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 61

Met Pro Gly Leu Ala Val Thr Ser Pro Thr Trp Val Val Arg Phe Thr  
1 5 10 15

Asn Asn Pro Val Thr Ala Ala Ser Arg Met Asp His Ala Ala Trp Leu  
20 25 30

Val Leu Ile Ser Gly Ser Ser Leu Glu Leu Arg Leu Arg Ser Ala  
35 40 45

&lt;210&gt; 62

&lt;211&gt; 51

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (51)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 62

Leu Leu Val Asp Phe Asp Ser Asp Lys Arg Thr Gln Pro Ile Trp Val  
1 5 10 15

Phe Phe Ala Leu Ile Val Trp Gly Phe Glu Val Leu Ala Arg Ser Trp  
20 25 30

Arg Gly Val Cys Val Gln Val Cys Glu Cys Met Cys Asn Val Cys Val  
35 40 45

Gln Val Xaa  
50

&lt;210&gt; 63

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (54)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 63

Met Gly Ser Ser Thr Phe Ile Leu Phe Phe Ser Phe Leu Leu Phe Ser  
1 5 10 15

Pro Phe Leu Ser Pro Ser Pro Thr Thr Ser Leu Tyr Phe Phe Phe Ile  
20 25 30

Tyr Ser Arg Leu Ile Phe Ser Ala Lys Arg Cys Leu Gly Pro Leu Thr  
35 40 45

Ser Gln Glu Ala Leu Xaa

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34

50

<210> 64  
<211> 21  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (21)  
<223> Xaa equals stop translation

<400> 64  
Met Gly Ala Thr Ala Arg Pro Gly Pro Thr Arg Gly Arg Trp Asp Ser  
1 5 10 15

Cys Leu Arg Gln Xaa  
20

<210> 65  
<211> 32  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (32)  
<223> Xaa equals stop translation

<400> 65  
Met Val Gly Pro Arg Leu Pro Met Gly Leu Leu Phe Val Ala Ser Phe  
1 5 10 15

Leu Phe Leu Phe Ile Gln Cys Val His Met Pro Arg Ser Tyr Leu Xaa  
20 25 30

<210> 66  
<211> 39  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (39)  
<223> Xaa equals stop translation

<400> 66  
Met Trp Leu Ile Leu Leu Ala Ile Pro Gly Val Ala Ala His Ala Trp  
1 5 10 15

Leu Val Trp Val His Arg Gly Ser His Gly Trp Ala Gln Gln Arg Gly  
20 25 30



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Pro Ser Gly Glu Gly Phe Xaa  
35

<210> 67  
<211> 32  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (32)  
<223> Xaa equals stop translation

<400> 67  
Met Val Cys Trp Leu Leu Pro Leu Trp Val Thr Val Leu Ser Phe Pro  
1 5 10 15  
  
Val Gly Arg Asp Val Ala Ala Leu Leu Ile Phe Thr Ser Ser Tyr Xaa  
20 25 30

<210> 68  
<211> 6  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (6)  
<223> Xaa equals stop translation

<400> 68  
Met Pro Cys Leu Phe Xaa  
1 5

<210> 69  
<211> 74  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (74)  
<223> Xaa equals stop translation

<400> 69  
Met Gly Cys Phe Val Val Val Val Phe Val Val Gly His Leu Ser  
1 5 10 15  
  
Ile Phe Ser His Gln Phe Cys Ser Leu Leu Ala Met Asp Lys Ile Gly  
20 25 30

Ser Phe Trp Leu Ile Lys Lys Asp Asn Phe Ile Lys Trp His Phe Lys  
35 40 45

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Gln Ala Ile Val Ser Phe Ile Ile Cys Asn Ala His Gly Lys Ala Lys  
50 55 60

Thr Phe Val Met Lys Glu Leu Leu Ile Xaa  
65 70

<210> 70  
<211> 2  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (2)  
<223> Xaa equals stop translation

<400> 70  
Ile Xaa  
1

<210> 71  
<211> 40  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (40)  
<223> Xaa equals stop translation

<400> 71  
Met Tyr Ser Leu Ala Ser Ile Met Gln Ile Lys Lys Tyr Phe Ser Leu  
1 5 10 15

Ser Val Phe Leu Ala Leu Val Ser Ser Cys Cys Phe Cys Leu Asp Leu  
20 25 30

Glu Arg Leu Gln Leu Ser Asn Xaa  
35 40

<210> 72  
<211> 27  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (27)  
<223> Xaa equals stop translation

<400> 72  
Met Ile Arg Tyr Leu Leu Met Cys Pro Leu Tyr Ile Gln Leu Leu Phe  
1 5 10 15

Leu Thr Leu Leu Phe Val Leu Ser Leu Pro Xaa

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20

25

<210> 73  
<211> 37  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (37)  
<223> Xaa equals stop translation

<400> 73  
Leu Pro Ser Leu Leu Leu Phe Leu Asn Pro Phe Ile Phe Thr Ile Leu  
1 5 10 15

Leu Leu Phe Pro Cys Gly Gly Phe Asn Thr Ser Ile Phe Phe Leu Asn  
20 25 30

Gln Leu Asp Ser Xaa  
35

<210> 74  
<211> 41  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (41)  
<223> Xaa equals stop translation

<400> 74  
Gly Gly Ile Tyr Lys Phe Thr Leu Thr Val Cys Ala Ala His Ile Arg  
1 5 10 15

Phe Leu Val Leu Gly Leu Phe Leu Tyr Ser Leu His Gly Asp Trp Gln  
20 25 30

His Gln His His Met Ser Thr Thr Xaa  
35 40

<210> 75  
<211> 47  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (47)  
<223> Xaa equals stop translation

<400> 75  
Met Arg Leu Gly Ile Ser Glu Leu Ser Phe Leu Leu Gly Ala Leu Gly  
1 5 10 15

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Tyr Leu Ser Leu Pro Arg Val Gly Pro Leu Glu Pro Phe Leu Pro Trp  
20 25 30

Val Gly Ala Val Ser Gly Cys Trp Glu Gly Leu Cys Trp Pro Xaa  
35 40 45

<210> 76  
<211> 29  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (29)  
<223> Xaa equals stop translation

<400> 76  
Met Lys Lys Arg Leu Ser Pro Leu Ser Trp Ala Arg Cys Cys Leu Cys  
1 5 10 15

Phe Trp Leu Gln Trp Gly Arg Arg Thr Asn Arg Leu Xaa  
20 25

<210> 77  
<211> 31  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (31)  
<223> Xaa equals stop translation

<400> 77  
Met Trp Cys Met Leu Asp Ile Thr Phe Leu Leu Gly Leu Trp Asn Phe  
1 5 10 15

Gly Ile Cys Gln Ala Glu Gly Ile Asn Val Thr Thr Pro Gln Xaa  
20 25 30

<210> 78  
<211> 38  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (38)  
<223> Xaa equals stop translation

<400> 78  
Met Lys Ile Leu Leu Cys Leu Cys Leu Ile Leu Leu Arg Val Arg  
1 5 10 15

Ser Cys Arg Arg Lys Ala Ala Arg Ala Leu Gly Met Glu Ala Ala  
20 25 30

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Asp Ala Val Thr Asp Xaa  
35

&lt;210&gt; 79

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (41)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 79

Met Thr Ala Val Gly Gly Ser Ile Cys Val Met Leu Val Val Ile Cys  
1 5 10 15

Leu Leu Val Ala Tyr Ile Thr Glu Asn Leu Met Arg Pro Ala Leu Ala  
20 25 30

Arg Pro Gly Leu Arg Arg His Pro Xaa  
35 40

&lt;210&gt; 80

&lt;211&gt; 77

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (77)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 80

Met Gly Lys Pro Pro Thr His Glu Ala Ile His Ser Val Val Lys Trp  
1 5 10 15

Leu Leu Ile Val Thr Lys Leu Leu Arg Leu Ser Gln Ile Cys Phe Leu  
20 25 30

Leu Phe Pro Leu Phe Leu Phe Leu Thr Ser Gly Thr Arg Leu Gly Ile  
35 40 45

Arg Leu Ile Phe Phe Cys Lys Thr Ala Glu Phe Phe Ile Phe Asn Ile  
50 55 60

His Phe Ile Ile Arg Lys Ile Ile Tyr Thr Ile Ser Xaa  
65 70 75

&lt;210&gt; 81

&lt;211&gt; 72

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

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<221> SITE  
<222> (72)  
<223> Xaa equals stop translation

<400> 81  
Met Ile Met Ser Ser Ser Asn Ser Ser Ile Trp Pro Ala Met Pro Cys  
1 5 10 15  
Met Leu Leu Ser Val Tyr Ser Tyr Leu Phe Ser Cys Ile Ser His Gly  
20 25 30  
Ser Pro Arg Leu Ser Ala Leu Gln Pro Pro Lys Pro Cys Ser Ser Phe  
35 40 45  
Ser Asn Ser Ser Ala Pro Tyr Leu Arg Ala Phe Ala Ser Ala Arg Cys  
50 55 60  
Thr Ser Phe Pro Tyr Phe Thr Xaa  
65 70

<210> 82  
<211> 5  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (5)  
<223> Xaa equals stop translation

<400> 82  
Met His Ala Leu Xaa  
1 5

<210> 83  
<211> 35  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (35)  
<223> Xaa equals stop translation

<400> 83  
Met Trp Pro Leu Ile Gly Tyr Leu Thr Lys Ser Leu Leu Asn Phe Ile  
1 5 10 15  
Leu Cys Val Ile Ile Ala Met Cys Leu Cys Val Leu Ser Ser Ser Asp  
20 25 30  
Thr Tyr Xaa  
35

<210> 84  
<211> 28

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<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (8)  
<223> Xaa equals any of the naturally occurring L-amino acids

<220>  
<221> SITE  
<222> (28)  
<223> Xaa equals stop translation

<400> 84  
Val Phe Trp Pro Thr Ser Glu Xaa Leu Leu Asn Cys Met Val Trp Gly  
1 5 10 15

Arg Glu Gly Asn Leu Lys Ser Arg Pro Asn Lys Xaa  
20 25

<210> 85  
<211> 35  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (35)  
<223> Xaa equals stop translation

<400> 85  
Met Leu Val Thr Glu Phe Cys Ile Val Leu Leu Phe Phe Leu Phe Gln  
1 5 10 15

Tyr Ile Gln Phe Asp Asp Val Leu Glu Ile Gly Ala Asn Ile Tyr Thr  
20 25 30

Pro Leu Xaa  
35

<210> 86  
<211> 32  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (32)  
<223> Xaa equals stop translation

<400> 86  
Met Ala Leu Phe Arg Ile Phe Leu Val Ala Trp Phe Val Ala Phe Leu  
1 5 10 15

Ser Val Arg Lys Leu Asn Pro Ile Leu Arg Ala Leu Glu Glu Leu Xaa  
20 25 30

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<210> 87  
<211> 33  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (33)  
<223> Xaa equals stop translation

<400> 87  
Met Phe Leu Leu Phe Leu Arg Ser Leu Pro Tyr Ile Leu Cys Ile Val  
1 5 10 15  
Lys Pro Phe Leu Lys Ser Asn Asp Leu Gly Lys Pro Thr Leu Val Cys  
20 25 30

Xaa

<210> 88  
<211> 22  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (22)  
<223> Xaa equals stop translation

<400> 88  
Met Thr His Tyr Val Phe Leu Leu Leu Phe Met Val Gly Arg Gly Asp  
1 5 10 15  
Glu Gly Lys Ser Gly Xaa  
20

<210> 89  
<211> 51  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (51)  
<223> Xaa equals stop translation

<400> 89  
Met Pro Thr His Pro Thr Arg Leu Val Leu Leu Pro Ile Asp Gly  
1 5 10 15  
Glu His Leu Ala Gly Ser Leu Leu Ile Ser Thr Val Leu Leu Gly Pro  
20 25 30



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Gly Thr Leu Gln Leu Pro Cys Lys Cys Ser Ala Gln Arg Leu Pro Asn  
35 40 45

Thr Ser Xaa  
50

&lt;210&gt; 90

&lt;211&gt; 59

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (59)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 90

Met Ser His Arg Ala Gln Pro Thr Phe Thr Ser Lys Val Tyr Val Met  
1 5 10 15

Val Val Ile Phe Ser Leu Leu Glu Tyr Ile Lys Trp Leu Ile Trp Gly  
20 25 30

Gly Lys Leu Ile Leu Asn Ile Asn Gly Gly Glu Lys Gly Thr Val Phe  
35 40 45

Thr Leu Lys Cys Lys Ser Glu His Thr Lys Xaa  
50 55

&lt;210&gt; 91

&lt;211&gt; 30

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (30)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 91

Met Leu Cys Val Leu Ser Ser Phe Leu Leu Ile Phe Leu Phe Leu Ile  
1 5 10 15

Cys Val Gln Phe Cys Ala Ala Leu Leu Ala Leu Leu Leu Xaa  
20 25 30

&lt;210&gt; 92

&lt;211&gt; 54

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (54)

&lt;223&gt; Xaa equals stop translation

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&lt;400&gt; 92

Met Ser Asn Leu Ser Leu Arg Tyr Ser Ser Ser Lys Gly Ser Trp Gly  
 1 5 10 15

Ser Leu Phe Pro Asn Ile Arg Ile Met Gln Ile Phe Leu Leu Met Val  
 20 25 30

Asp Ala His Cys Val Tyr Ile Ser Gln Pro Phe Lys His Glu Ser Glu  
 35 40 45

Lys Ser Phe Ser Lys Xaa  
 50

&lt;210&gt; 93

&lt;211&gt; 92

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (92)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 93

Met Asn Lys Ser Thr Phe Leu Phe Leu Ile Ile Met Trp Pro Asn Val  
 1 5 10 15

Tyr Ile Ser Leu Ser Val Ala His His Ile Val Leu Thr Lys Thr Ile  
 20 25 30

Ser Ser Phe Thr Pro Ala Gln Ile Phe Lys Tyr Ser Leu Leu Phe Pro  
 35 40 45

Thr Ser Val Ser Gly Ile Ala Ile Leu Phe Lys Thr Tyr Leu Lys Asn  
 50 55 60

Val Gln Ala Ser His Leu Cys Val Lys Leu Phe Ser Leu Phe Gln Cys  
 65 70 75 80

Asp Glu Lys Asn Trp Thr Glu His Thr Phe Leu Xaa  
 85 90

&lt;210&gt; 94

&lt;211&gt; 44

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (44)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 94

Met Pro Leu Leu Phe Phe Ser Val Ser Thr Leu Phe Ser Gly Ser Val  
 1 5 10 15

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Thr Leu Gln Gln Arg Gly Met Phe Leu Pro Trp Thr Gly Thr Gly Asn  
20 25 30

Arg Cys Leu Pro Cys Tyr Gly His Gly Leu Asn Xaa  
35 40

&lt;210&gt; 95

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (32)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 95

Met Phe Leu Asp Phe Cys Trp Gln Ala Cys Ser Ser Ile Cys Met Ala  
1 5 10 15

Pro Ser Pro His Leu His Phe Ser Ala Gln Met Leu Pro Ser Pro Xaa  
20 25 30

&lt;210&gt; 96

&lt;211&gt; 25

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (25)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 96

Cys Thr Thr Val Val Leu Phe Leu Leu Ile Glu Leu Lys Gln Leu Tyr  
1 5 10 15

Gly Ala Lys Val Thr Gly Tyr Ser Xaa  
20 25

&lt;210&gt; 97

&lt;211&gt; 41

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (41)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 97

Met Cys Lys Met Phe Asn Leu Pro Phe Leu Leu Val Ser Val Ala  
1 5 10 15

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Leu Trp Cys Lys Gln Leu Lys Thr Leu Asn Val Tyr Thr Ile Arg Pro  
                   20                  25                  30

Arg Arg Gln Met Lys Tyr Phe Phe Xaa  
           35                  40

<210> 98  
 <211> 34  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (34)  
 <223> Xaa equals stop translation

<400> 98  
 Met Gly Leu Leu Trp Asn Leu Ile Ala Thr Tyr Phe Cys Phe Pro Leu  
       1                  5                  10                  15

Asp Ala Ala Ser Thr His Val Asp Tyr Glu Val Leu Thr His Pro Arg  
                   20                  25                  30

Ser Xaa

<210> 99  
 <211> 48  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (48)  
 <223> Xaa equals stop translation

<400> 99  
 Met Tyr Ser Lys Ser Ser Ala Asn Ile Ser Gln Gly Met Leu Asp Ile  
       1                  5                  10                  15

Leu Leu Trp Ile Asp Leu Ala His Asp Asp Leu Leu Gly Ser Pro Ser  
                   20                  25                  30

Pro Val Ile Leu Arg Ser His Ala His Ser Gly Ile Leu Thr Val Xaa  
           35                  40                  45

<210> 100  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<220>

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&lt;221&gt; SITE

&lt;222&gt; (10)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 100

Ser Cys Ser Ile Arg Tyr Asp Leu Phe Xaa  
 1 5 10

&lt;210&gt; 101

&lt;211&gt; 289

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 101

Glu Ile Gln Lys Lys Gly Lys Thr Ser Ser Gly Thr Leu Ile Ser Thr  
 1 5 10 15

Ser Ser Lys Ser Leu Met Ala Lys Ser Val Gln Ser Gln Gln Gln  
 20 25 30

Gly Gln Leu Arg Arg Thr His Ser Glu Gly Asp Gly Ala Glu Arg Ala  
 35 40 45

Lys Ser Arg Ser Asn Ala Ile Asp Lys Ala Thr Glu Thr Thr Leu Glu  
 50 55 60

Thr Pro Leu Thr Ile Ala Cys Ala Asn Gly His Lys Asp Ile Val Glu  
 65 70 75 80

Leu Leu Leu Lys Glu Gly Ala Asn Ile Glu His Arg Asp Lys Lys Gly  
 85 90 95

Phe Ser Pro Leu Ile Ile Ala Ala Thr Ala Gly His Ser Ser Val Val  
 100 105 110

Glu Val Leu Leu Lys Asn His Ala Ala Ile Glu Ala Gln Ser Asp Arg  
 115 120 125

Thr Lys Asp Thr Ala Leu Ser Leu Ala Cys Ser Gly Gly Arg Lys Asp  
 130 135 140

Val Val Glu Leu Leu Leu Ala His Gly Ala Asn Lys Glu His Arg Asn  
 145 150 155 160

Val Ser Asp Tyr Thr Pro Leu Ser Leu Ala Ser Ser Gly Gly Tyr Ile  
 165 170 175

Glu Ile Val Asn Met Leu Leu Thr Ala Gly Ser Glu Ile Asn Ser Arg  
 180 185 190

Thr Gly Ser Lys Leu Gly Ile Ser Pro Leu Met Leu Ala Ser Met Asn  
 195 200 205

Gly His Arg Glu Ala Thr Arg Val Leu Leu Glu Lys Gly Ser Asp Ile  
 210 215 220

Asn Ala Gln Ile Glu Thr Asn Arg Asn Thr Ala Leu Thr Leu Ala Ser  
 225 230 235 240

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Phe Gln Gly Arg Thr Glu Val Val Lys Leu Leu Leu Ala Tyr Asn Ala  
 245 250 255

Asn Val Glu His Arg Ala Lys Thr Gly Leu Thr Pro Leu Met Glu Cys  
 260 265 270

Ala Ser Gly Gly Tyr Val Asp Val Gly Asn Leu Leu Ile Ala Ala Gly  
 275 280 285

Ala

<210> 102  
 <211> 293  
 <212> PRT  
 <213> Homo sapiens

<400> 102  
 Glu Val Leu Arg Arg Leu Thr Ser Ser Val Ser Cys Ala Leu Asp Glu  
 1 5 10 15

Ala Ala Ala Ala Leu Thr Arg Met Arg Ala Glu Ser Thr Ala Asn Ala  
 20 25 30

Gly Gln Ser Asp Asn Arg Ser Leu Ala Glu Ala Cys Ser Glu Gly Asp  
 35 40 45

Val Asn Ala Val Arg Lys Leu Leu Ile Glu Gly Arg Ser Val Asn Glu  
 50 55 60

His Thr Glu Glu Gly Glu Ser Leu Leu Cys Leu Ala Cys Ser Ala Gly  
 65 70 75 80

Tyr Tyr Glu Leu Ala Gln Val Leu Leu Ala Met His Ala Asn Val Glu  
 85 90 95

Asp Arg Gly Ile Lys Gly Asp Ile Thr Pro Leu Met Ala Ala Ala Asn  
 100 105 110

Gly Gly His Val Lys Ile Val Lys Leu Leu Leu Ala His Lys Ala Asp  
 115 120 125

Val Asn Ala Gln Ser Ser Thr Gly Asn Thr Ala Leu Thr Tyr Ala Cys  
 130 135 140

Ala Gly Gly Tyr Val Asp Val Val Lys Val Leu Leu Glu Ser Gly Ala  
 145 150 155 160

Ser Ile Glu Asp His Asn Glu Asn Gly His Thr Pro Leu Met Glu Ala  
 165 170 175

Gly Ser Ala Gly His Val Glu Val Ala Arg Leu Leu Leu Glu Asn Gly  
 180 185 190

Ala Gly Ile Asn Thr His Ser Asn Glu Phe Lys Glu Ser Ala Leu Thr  
 195 200 205

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Leu Ala Cys Tyr Lys Gly His Leu Glu Met Val Arg Phe Leu Leu Glu  
 210 215 220  
 Ala Gly Ala Asp Gln Glu His Lys Thr Asp Glu Met His Thr Ala Leu  
 225 230 235 240  
 Met Glu Ala Cys Met Asp Gly His Val Glu Val Ala Arg Leu Leu Leu  
 245 250 255  
 Asp Ser Gly Ala Gln Val Asn Met Pro Ala Asp Ser Phe Glu Ser Pro  
 260 265 270  
 Leu Thr Leu Ala Ala Cys Gly Gly His Val Glu Leu Ala Ala Leu Leu  
 275 280 285  
 Ile Glu Arg Gly Ala  
 290

&lt;210&gt; 103

&lt;211&gt; 289

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 103

Glu Ile Gln Lys Lys Gly Lys Thr Ser Ser Gly Thr Leu Ile Ser Thr  
 1 5 10 15  
 Ser Ser Lys Ser Leu Met Ala Lys Ser Val Gln Ser Gln Gln Gln  
 20 25 30  
 Gly Gln Leu Arg Arg Thr His Ser Glu Gly Asp Gly Ala Glu Arg Ala  
 35 40 45  
 Lys Ser Arg Ser Asn Ala Ile Asp Lys Ala Thr Glu Thr Thr Leu Glu  
 50 55 60  
 Thr Pro Leu Thr Ile Ala Cys Ala Asn Gly His Lys Asp Ile Val Glu  
 65 70 75 80  
 Leu Leu Leu Lys Glu Gly Ala Asn Ile Glu His Arg Asp Lys Lys Gly  
 85 90 95  
 Phe Ser Pro Leu Ile Ile Ala Ala Thr Ala Gly His Ser Ser Val Val  
 100 105 110  
 Glu Val Leu Leu Lys Asn His Ala Ala Ile Glu Ala Gln Ser Asp Arg  
 115 120 125  
 Thr Lys Asp Thr Ala Leu Ser Leu Ala Cys Ser Gly Gly Arg Lys Asp  
 130 135 140  
 Val Val Glu Leu Leu Leu Ala His Gly Ala Asn Lys Glu His Arg Asn  
 145 150 155 160  
 Val Ser Asp Tyr Thr Pro Leu Ser Leu Ala Ser Ser Gly Gly Tyr Ile  
 165 170 175  
 Glu Ile Val Asn Met Leu Leu Thr Ala Gly Ser Glu Ile Asn Ser Arg

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180                      185                      190  
 Thr Gly Ser Lys Leu Gly Ile Ser Pro Leu Met Leu Ala Ser Met Asn  
 195                      200                      205  
 Gly His Arg Glu Ala Thr Arg Val Leu Leu Glu Lys Gly Ser Asp Ile  
 210                      215                      220  
 Asn Ala Gln Ile Glu Thr Asn Arg Asn Thr Ala Leu Thr Leu Ala Ser  
 225                      230                      235                      240  
 Phe Gln Gly Arg Thr Glu Val Val Lys Leu Leu Ala Tyr Asn Ala  
 245                      250                      255  
 Asn Val Glu His Arg Ala Lys Thr Gly Leu Thr Pro Leu Met Glu Cys  
 260                      265                      270  
 Ala Ser Gly Gly Tyr Val Asp Val Gly Asn Leu Leu Ile Ala Ala Gly  
 275                      280                      285

Ala

<210> 104  
 <211> 293  
 <212> PRT  
 <213> Homo sapiens

<400> 104  
 Glu Val Leu Arg Arg Leu Thr Ser Ser Val Ser Cys Ala Leu Asp Glu  
 1                      5                      10                      15  
 Ala Ala Ala Ala Leu Thr Arg Met Arg Ala Glu Ser Thr Ala Asn Ala  
 20                      25                      30  
 Gly Gln Ser Asp Asn Arg Ser Leu Ala Glu Ala Cys Ser Glu Gly Asp  
 35                      40                      45  
 Val Asn Ala Val Arg Lys Leu Leu Ile Glu Gly Arg Ser Val Asn Glu  
 50                      55                      60  
 His Thr Glu Glu Gly Glu Ser Leu Leu Cys Leu Ala Cys Ser Ala Gly  
 65                      70                      75                      80  
 Tyr Tyr Glu Leu Ala Gln Val Leu Leu Ala Met His Ala Asn Val Glu  
 85                      90                      95  
 Asp Arg Gly Ile Lys Gly Asp Ile Thr Pro Leu Met Ala Ala Asn  
 100                      105                      110  
 Gly Gly His Val Lys Ile Val Lys Leu Leu Leu Ala His Lys Ala Asp  
 115                      120                      125  
 Val Asn Ala Gln Ser Ser Thr Gly Asn Thr Ala Leu Thr Tyr Ala Cys  
 130                      135                      140  
 Ala Gly Gly Tyr Val Asp Val Val Lys Val Leu Leu Glu Ser Gly Ala  
 145                      150                      155                      160



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Ser Ile Glu Asp His Asn Glu Asn Gly His Thr Pro Leu Met Glu Ala  
 165 170 175

Gly Ser Ala Gly His Val Glu Val Ala Arg Leu Leu Leu Glu Asn Gly  
 180 185 190

Ala Gly Ile Asn Thr His Ser Asn Glu Phe Lys Glu Ser Ala Leu Thr  
 195 200 205

Leu Ala Cys Tyr Lys Gly His Leu Glu Met Val Arg Phe Leu Leu Glu  
 210 215 220

Ala Gly Ala Asp Gln Glu His Lys Thr Asp Glu Met His Thr Ala Leu  
 225 230 235 240

Met Glu Ala Cys Met Asp Gly His Val Glu Val Ala Arg Leu Leu Leu  
 245 250 255

Asp Ser Gly Ala Gln Val Asn Met Pro Ala Asp Ser Phe Glu Ser Pro  
 260 265 270

Leu Thr Leu Ala Ala Cys Gly Gly His Val Glu Leu Ala Ala Leu Leu  
 275 280 285

Ile Glu Arg Gly Ala  
 290

&lt;210&gt; 105

&lt;211&gt; 96

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 105

Thr Asn Glu Val Pro Glu Val Thr Val Phe Ser Lys Ser Pro Val Thr  
 1 5 10 15

Leu Gly Gln Pro Asn Thr Leu Ile Cys Leu Val Asp Asn Ile Phe Pro  
 20 25 30

Pro Val Val Asn Ile Thr Trp Leu Ser Asn Gly Gln Ser Val Thr Glu  
 35 40 45

Gly Val Ser Glu Thr Ser Phe Leu Ser Lys Ser Asp His Ser Phe Phe  
 50 55 60

Lys Ile Ser Tyr Leu Thr Phe Leu Pro Ser Ala Asp Glu Ile Tyr Asp  
 65 70 75 80

Cys Lys Val Glu His Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp  
 85 90 95

&lt;210&gt; 106

&lt;211&gt; 96

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&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 106

Thr Thr Glu Val Pro Glu Val Thr Val Phe Ser Lys Ser Pro Val Thr  
1 5 10 15

Leu Gly Gln Pro Asn Thr Leu Ile Cys Leu Val Asp Asn Ile Phe Pro  
20 25 30

Pro Val Val Asn Ile Thr Trp Leu Ser Asn Gly His Ala Val Thr Glu  
35 40 45

Gly Val Ser Glu Thr Ser Phe Leu Ser Lys Ser Asp His Ser Phe Phe  
50 55 60

Lys Ile Ser Tyr Leu Thr Phe Leu Pro Ser Ala Asp Glu Ile Tyr Asp  
65 70 75 80

Cys Lys Val Glu His Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp  
85 90 95

&lt;210&gt; 107

&lt;211&gt; 83

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 107

Ala Asp His Val Ala Ser Cys Gly Val Asn Leu Tyr Gln Phe Tyr Gly  
1 5 10 15

Pro Ser Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Gln Phe Tyr  
20 25 30

Val Asp Leu Glu Arg Lys Glu Thr Ala Trp Arg Trp Pro Glu Phe Ser  
35 40 45

Lys Phe Gly Gly Phe Asp Pro Gln Gly Ala Leu Arg Asn Met Ala Val  
50 55 60

Ala Lys His Asn Leu Asn Ile Met Ile Lys Arg Tyr Asn Ser Thr Ala  
65 70 75 80

Ala Thr Asn

&lt;210&gt; 108

&lt;211&gt; 83

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 108

Ala Asp His Val Ala Ser Cys Gly Val Asn Leu Tyr Gln Phe Tyr Gly  
1 5 10 15

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Pro Ser Gly Gln Phe Thr His Glu Phe Asp Gly Asp Glu Gln Phe Tyr  
                   20                                  25                                  30

Val Asp Leu Glu Lys Lys Glu Thr Ala Trp Arg Trp Pro Glu Phe Ser  
                   35                                  40                                  45

Lys Phe Gly Gly Phe Asp Pro Gln Gly Ala Leu Arg Asn Met Ala Val  
                   50                                  55                                  60

Ala Lys His Asn Leu Asn Ile Met Ile Lys Arg Tyr Asn Ser Thr Ala  
                   65                                  70                                  75                                  80

Ala Thr Asn

<210> 109  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<400> 109  
 Val Cys Ala Leu Gly Leu Ser Val Gly Leu Met Gly Ile Val Val Gly  
                   1                                  5                                  10                                  15

Thr Val Phe Ile Ile Gln Gly Leu Arg Ser Val Gly Ala Ser Arg His  
                   20                                  25                                  30

Gln Gly Pro Leu  
                   35

<210> 110  
 <211> 36  
 <212> PRT  
 <213> Homo sapiens

<400> 110  
 Leu Cys Pro Leu Gly Leu Leu Trp Ala Ser Leu Gly Ile Val Val Gly  
                   1                                  5                                  10                                  15

Thr Val Phe Ile Ile Gln Gly Leu Arg Ser Val Gly Ala Ser Arg His  
                   20                                  25                                  30

Gln Gly Pro Leu  
                   35

<210> 111  
 <211> 29  
 <212> PRT  
 <213> Homo sapiens

<400> 111  
 Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp Glu Pro Glu Ile Pro  
                   1                                  5                                  10                                  15

Ala Pro Met Ser Glu Leu Thr Glu Thr Val Val Cys Ala

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20

25

&lt;210&gt; 112

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 112

Trp His Ser Asp Leu Asn Asn Phe Thr Ser Thr Glu Pro Glu Ile Pro  
 1 5 10 15

Ala Pro Met Ser Glu Leu Thr Glu Thr Val Val Cys Ala  
 20 25

&lt;210&gt; 113

&lt;211&gt; 96

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 113

Thr Asn Glu Val Pro Glu Val Thr Val Phe Ser Lys Ser Pro Val Thr  
 1 5 10 15

Leu Gly Gln Pro Asn Thr Leu Ile Cys Leu Val Asp Asn Ile Phe Pro  
 20 25 30

Pro Val Val Asn Ile Thr Trp Leu Ser Asn Gly Gln Ser Val Thr Glu  
 35 40 45

Gly Val Ser Glu Thr Ser Phe Leu Ser Lys Ser Asp His Ser Phe Phe  
 50 55 60

Lys Ile Ser Tyr Leu Thr Phe Leu Pro Ser Ala Asp Glu Ile Tyr Asp  
 65 70 75 80

Cys Lys Val Glu His Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp  
 85 90 95

&lt;210&gt; 114

&lt;211&gt; 96

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 114

Thr Thr Glu Val Pro Glu Val Thr Val Phe Ser Lys Ser Pro Val Thr  
 1 5 10 15

Leu Gly Gln Pro Asn Thr Leu Ile Cys Leu Val Asp Asn Ile Phe Pro  
 20 25 30

Pro Val Val Asn Ile Thr Trp Leu Ser Asn Gly His Ala Val Thr Glu  
 35 40 45

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Gly Val Ser Glu Thr Ser Phe Leu Ser Lys Ser Asp His Ser Phe Phe  
 50 55 60

Lys Ile Ser Tyr Leu Thr Phe Leu Pro Ser Ala Asp Glu Ile Tyr Asp  
 65 70 75 80

Cys Lys Val Glu His Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp  
 85 90 95

<210> 115  
 <211> 83  
 <212> PRT  
 <213> Homo sapiens

<400> 115  
 Ala Asp His Val Ala Ser Cys Gly Val Asn Leu Tyr Gln Phe Tyr Gly  
 1 5 10 15

Pro Ser Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu Gln Phe Tyr  
 20 25 30

Val Asp Leu Glu Arg Lys Glu Thr Ala Trp Arg Trp Pro Glu Phe Ser  
 35 40 45

Lys Phe Gly Gly Phe Asp Pro Gln Gly Ala Leu Arg Asn Met Ala Val  
 50 55 60

Ala Lys His Asn Leu Asn Ile Met Ile Lys Arg Tyr Asn Ser Thr Ala  
 65 70 75 80

Ala Thr Asn

<210> 116  
 <211> 83  
 <212> PRT  
 <213> Homo sapiens

<400> 116  
 Ala Asp His Val Ala Ser Cys Gly Val Asn Leu Tyr Gln Phe Tyr Gly  
 1 5 10 15

Pro Ser Gly Gln Phe Thr His Glu Phe Asp Gly Asp Glu Gln Phe Tyr  
 20 25 30

Val Asp Leu Glu Lys Lys Glu Thr Ala Trp Arg Trp Pro Glu Phe Ser  
 35 40 45

Lys Phe Gly Gly Phe Asp Pro Gln Gly Ala Leu Arg Asn Met Ala Val  
 50 55 60

Ala Lys His Asn Leu Asn Ile Met Ile Lys Arg Tyr Asn Ser Thr Ala  
 65 70 75 80

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Ala Thr Asn

&lt;210&gt; 117

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 117

Val Cys Ala Leu Gly Leu Ser Val Gly Leu Met Gly Ile Val Val Gly  
1 5 10 15

Thr Val Phe Ile Ile Gln Gly Leu Arg Ser Val Gly Ala Ser Arg His  
20 25 30

Gln Gly Pro Leu  
35

&lt;210&gt; 118

&lt;211&gt; 36

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 118

Leu Cys Pro Leu Gly Leu Leu Trp Ala Ser Leu Gly Ile Val Val Gly  
1 5 10 15

Thr Val Phe Ile Ile Gln Gly Leu Arg Ser Val Gly Ala Ser Arg His  
20 25 30

Gln Gly Pro Leu  
35

&lt;210&gt; 119

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 119

Trp Gly Leu Asp Gln Pro Leu Leu Lys His Trp Glu Pro Glu Ile Pro  
1 5 10 15

Ala Pro Met Ser Glu Leu Thr Glu Thr Val Val Cys Ala  
20 25

&lt;210&gt; 120

&lt;211&gt; 29

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 120

Trp His Ser Asp Leu Asn Asn Phe Thr Ser Thr Glu Pro Glu Ile Pro  
1 5 10 15

Ala Pro Met Ser Glu Leu Thr Glu Thr Val Val Cys Ala

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20

25

<210> 121  
<211> 81  
<212> PRT  
<213> Homo sapiens  
  
<400> 121  
Cys Ala Lys Cys Cys Val Arg Val His Ala Ser Cys Tyr Gly Ile Pro  
1 5 10 15  
Ser His Glu Ile Cys Asp Gly Trp Leu Cys Ala Arg Cys Lys Arg Asn  
20 25 30  
Ala Trp Thr Ala Glu Cys Cys Leu Cys Asn Leu Arg Gly Gly Ala Leu  
35 40 45  
Lys Gln Thr Lys Asn Asn Lys Trp Ala His Val Met Cys Ala Val Ala  
50 55 60  
Val Pro Glu Val Arg Phe Thr Asn Val Pro Glu Arg Thr Gln Ile Asp  
65 70 75 80  
Val

<210> 122  
<211> 81  
<212> PRT  
<213> Homo sapiens  
  
<400> 122  
Cys Gly Lys Cys Cys Leu Gln Val His Ala Ser Cys Tyr Gly Ile Arg  
1 5 10 15  
Pro Glu Leu Val Asn Glu Gly Trp Thr Cys Ser Arg Cys Ala Ala His  
20 25 30  
Ala Trp Thr Ala Glu Cys Cys Leu Cys Asn Leu Arg Gly Gly Ala Leu  
35 40 45  
Gln Met Thr Thr Asp Arg Arg Trp Ile His Val Ile Cys Ala Ile Ala  
50 55 60  
Val Pro Glu Ala Arg Phe Leu Asn Val Ile Glu Arg His Pro Val Asp  
65 70 75 80  
Ile

<210> 123  
<211> 127  
<212> PRT  
<213> Homo sapiens  
  
<400> 123

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Gln Glu Tyr Asn Ala Thr Val Ala Arg Met Lys Pro His Cys Ala Ile  
 1 5 10 15  
 Cys Thr Leu Leu Met Pro Tyr His Lys Pro Asp Ser Ser Asn Glu Ala  
 20 25 30  
 Asn Asp Ala Arg Trp Glu Thr Lys Leu Asp Glu Val Val Thr Ser Glu  
 35 40 45  
 Gly Lys Thr Lys Pro Leu Ile Pro Glu Met Cys Phe Ile Tyr Ser Glu  
 50 55 60  
 Glu Asn Ile Glu Tyr Ser Pro Pro Asn Ala Phe Leu Glu Glu Asp Gly  
 65 70 75 80  
 Thr Ser Leu Leu Ile Ser Cys Ala Lys Cys Cys Val Arg Val His Ala  
 85 90 95  
 Ser Cys Tyr Gly Ile Pro Ser His Glu Ile Cys Asp Gly Trp Leu Cys  
 100 105 110  
 Ala Arg Cys Lys Arg Asn Ala Trp Thr Ala Glu Cys Cys Leu Cys  
 115 120 125

&lt;210&gt; 124

&lt;211&gt; 132

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (7)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (65)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 124

Glu Glu Val Gln Arg Ser Xaa Ala Arg Thr Glu Pro Tyr Cys Ala Ile  
 1 5 10 15  
 Cys Thr Leu Phe Tyr Pro Tyr Cys Gln Ala Leu Gln Thr Glu Lys Glu  
 20 25 30  
 Ala Pro Ile Ala Ser Leu Gly Glu Gly Cys Pro Ala Thr Leu Pro Ser  
 35 40 45  
 Lys Ser Arg Gln Lys Thr Arg Pro Leu Ile Pro Glu Met Cys Phe Thr  
 50 55 60  
 Xaa Gly Gly Glu Asn Thr Glu Pro Leu Pro Ala Asn Ser Tyr Ile Gly  
 65 70 75 80  
 Asp Asp Gly Thr Ser Pro Leu Ile Pro Ala Ala Ser Ala Ala Cys Arg  
 85 90 95



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Ser Met Pro Val Ala Met Ala Ser Val Pro Ser Trp Ser Met Lys Ala  
 100 105 110

Gly Arg Val Pro Gly Ala Arg Pro Thr Pro Gly Leu Arg Ser Ala Ala  
 115 120 125

Cys Ala Thr Cys  
 130

&lt;210&gt; 125

&lt;211&gt; 81

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 125

Cys Ala Lys Cys Cys Val Arg Val His Ala Ser Cys Tyr Gly Ile Pro  
 1 5 10 15

Ser His Glu Ile Cys Asp Gly Trp Leu Cys Ala Arg Cys Lys Arg Asn  
 20 25 30

Ala Trp Thr Ala Glu Cys Cys Leu Cys Asn Leu Arg Gly Gly Ala Leu  
 35 40 45

Lys Gln Thr Lys Asn Asn Lys Trp Ala His Val Met Cys Ala Val Ala  
 50 55 60

Val Pro Glu Val Arg Phe Thr Asn Val Pro Glu Arg Thr Gln Ile Asp  
 65 70 75 80

Val

&lt;210&gt; 126

&lt;211&gt; 81

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 126

Cys Gly Lys Cys Cys Leu Gln Val His Ala Ser Cys Tyr Gly Ile Arg  
 1 5 10 15

Pro Glu Leu Val Asn Glu Gly Trp Thr Cys Ser Arg Cys Ala Ala His  
 20 25 30

Ala Trp Thr Ala Glu Cys Cys Leu Cys Asn Leu Arg Gly Gly Ala Leu  
 35 40 45

Gln Met Thr Thr Asp Arg Arg Trp Ile His Val Ile Cys Ala Ile Ala  
 50 55 60

Val Pro Glu Ala Arg Phe Leu Asn Val Ile Glu Arg His Pro Val Asp  
 65 70 75 80

Ile

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60

&lt;210&gt; 127

&lt;211&gt; 127

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 127

Gln Glu Tyr Asn Ala Thr Val Ala Arg Met Lys Pro His Cys Ala Ile  
     1                    5                    10                    15  
 Cys Thr Leu Leu Met Pro Tyr His Lys Pro Asp Ser Ser Asn Glu Glu  
             20                    25                    30  
 Asn Asp Ala Arg Trp Glu Thr Lys Leu Asp Glu Val Val Thr Ser Glu  
             35                    40                    45  
 Gly Lys Thr Lys Pro Leu Ile Pro Glu Met Cys Phe Ile Tyr Ser Glu  
             50                    55                    60  
 Glu Asn Ile Glu Tyr Ser Pro Pro Asn Ala Phe Leu Glu Glu Asp Gly  
             65                    70                    75                    80  
 Thr Ser Leu Leu Ile Ser Cys Ala Lys Cys Cys Val Arg Val His Ala  
             85                    90                    95  
 Ser Cys Tyr Gly Ile Pro Ser His Glu Ile Cys Asp Gly Trp Leu Cys  
             100                    105                    110  
 Ala Arg Cys Lys Arg Asn Ala Trp Thr Ala Glu Cys Cys Leu Cys  
             115                    120                    125

&lt;210&gt; 128

&lt;211&gt; 132

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (7)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (65)

&lt;223&gt; Xaa equals any of the naturally occurring L-amino acids

&lt;400&gt; 128

Glu Glu Val Gln Arg Ser Xaa Ala Arg Thr Glu Pro Tyr Cys Ala Ile  
     1                    5                    10                    15  
 Cys Thr Leu Phe Tyr Pro Tyr Cys Gln Ala Leu Gln Thr Glu Lys Glu  
             20                    25                    30  
 Ala Pro Ile Ala Ser Leu Gly Glu Gly Cys Pro Ala Thr Leu Pro Ser  
             35                    40                    45  
 Lys Ser Arg Gln Lys Thr Arg Pro Leu Ile Pro Glu Met Cys Phe Thr  
             50                    55                    60

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Xaa Gly Gly Glu Asn Thr Glu Pro Leu Pro Ala Asn Ser Tyr Ile Gly  
 65 70 75 80  
 Asp Asp Gly Thr Ser Pro Leu Ile Pro Ala Ala Ser Ala Ala Cys Arg  
 85 90 95  
 Ser Met Pro Val Ala Met Ala Ser Val Pro Ser Trp Ser Met Lys Ala  
 100 105 110  
 Gly Arg Val Pro Gly Ala Arg Pro Thr Pro Gly Leu Arg Ser Ala Ala  
 115 120 125  
 Cys Ala Thr Cys  
 130

<210> 129  
 <211> 88  
 <212> PRT  
 <213> Homo sapiens

<400> 129  
 Lys Gln Lys Leu Ile Gln Lys Tyr Lys Glu Ala Met Ser Asn Lys Ala  
 1 5 10 15  
 Cys Lys Tyr Phe Asp Glu Gly Arg Gly Ser Cys Pro Phe Gly Glu Asn  
 20 25 30  
 Cys Phe Tyr Lys His Met Tyr Pro Asp Gly Arg Arg Glu Glu Pro Gln  
 35 40 45  
 Arg Gln Gln Val Gly Thr Ser Ser Arg Asn Pro Gly Gln Gln Arg Asn  
 50 55 60  
 His Phe Trp Glu Phe Phe Glu Glu Gly Ala Asn Ser Asn Pro Phe Asp  
 65 70 75 80  
 Asp Glu Glu Glu Ala Val Thr Phe  
 85

<210> 130  
 <211> 89  
 <212> PRT  
 <213> Homo sapiens

<400> 130  
 Glu Gln Lys Leu Ile Leu Lys Tyr Lys Glu Ala Met Ser Asn Lys Ala  
 1 5 10 15  
 Cys Arg Tyr Phe Asp Glu Gly Arg Gly Ser Cys Pro Phe Gly Gly Asn  
 20 25 30  
 Cys Phe Tyr Lys His Ala Tyr Pro Asp Gly Arg Arg Glu Glu Pro Gln  
 35 40 45  
 Arg Gln Lys Val Gly Thr Ser Ser Arg Tyr Arg Ala Gln Arg Arg Asn  
 50 55 60

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His Phe Trp Glu Leu Ile Glu Glu Arg Glu Asn Ser Asn Pro Phe Asp  
 65 70 75 80

Asn Asp Glu Glu Glu Val Val Thr Phe  
 85

&lt;210&gt; 131

&lt;211&gt; 88

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 131

Lys Gln Lys Leu Ile Gln Lys Tyr Lys Glu Ala Met Ser Asn Lys Ala  
 1 5 10 15

Cys Lys Tyr Phe Asp Glu Gly Arg Gly Ser Cys Pro Phe Gly Glu Asn  
 20 25 30

Cys Phe Tyr Lys His Met Tyr Pro Asp Gly Arg Arg Glu Glu Pro Gln  
 35 40 45

Arg Gln Gln Val Gly Thr Ser Ser Arg Asn Pro Gly Gln Gln Arg Asn  
 50 55 60

His Phe Trp Glu Phe Phe Glu Glu Gly Ala Asn Ser Asn Pro Phe Asp  
 65 70 75 80

Asp Glu Glu Glu Ala Val Thr Phe  
 85

&lt;210&gt; 132

&lt;211&gt; 89

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 132

Glu Gln Lys Leu Ile Leu Lys Tyr Lys Glu Ala Met Ser Asn Lys Ala  
 1 5 10 15

Cys Arg Tyr Phe Asp Glu Gly Arg Gly Ser Cys Pro Phe Gly Gly Asn  
 20 25 30

Cys Phe Tyr Lys His Ala Tyr Pro Asp Gly Arg Arg Glu Glu Pro Gln  
 35 40 45

Arg Gln Lys Val Gly Thr Ser Ser Arg Tyr Arg Ala Gln Arg Arg Asn  
 50 55 60

His Phe Trp Glu Leu Ile Glu Glu Arg Glu Asn Ser Asn Pro Phe Asp  
 65 70 75 80

Asn Asp Glu Glu Glu Val Val Thr Phe  
 85

&lt;210&gt; 133

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<211> 47  
<212> PRT  
<213> Homo sapiens

<400> 133  
Arg Leu Glu Cys Asn Gly Ala Ile Ser Ala His Arg Asn Leu Arg Leu  
1 5 10 15  
Pro Gly Ser Ser Asp Ser Pro Ala Ser Ala Ser Pro Val Ala Gly Ile  
20 25 30  
Thr Gly Met Cys Thr His Ala Arg Leu Ile Leu Tyr Phe Phe Leu  
35 40 45

<210> 134  
<211> 47  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (44)  
<223> Xaa equals stop translation

<400> 134  
Arg Leu Glu Cys Ser Gly Thr Ile Thr Ala His Cys Ser Leu Asn Leu  
1 5 10 15  
Thr Gly Ser Ile Asn Pro Pro Ile Pro Ala Ser Leu Ile Ala Gly Ala  
20 25 30  
Thr Gly Met His His His Ser Gln Leu Ile Ile Xaa Phe Phe Val  
35 40 45

<210> 135  
<211> 84  
<212> PRT  
<213> Homo sapiens

<400> 135  
Val Thr Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Leu Gln Pro  
1 5 10 15  
Leu Pro Pro Gly Phe Lys Leu Phe Ser Cys Pro Ser Leu Leu Ser Ser  
20 25 30  
Trp Asp Tyr Arg Arg Pro Pro Arg Leu Ala Asn Phe Phe Val Phe Leu  
35 40 45  
Val Glu Met Gly Phe Thr Met Phe Ala Arg Leu Ile Leu Ile Ser Gly  
50 55 60  
Pro Cys Asp Leu Pro Ala Ser Ala Ser Gln Ser Ala Gly Ile Thr Gly  
65 70 75 80  
Val Ser His His

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<210> 136  
<211> 86  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (15)  
<223> Xaa equals stop translation

<220>  
<221> SITE  
<222> (67)  
<223> Xaa equals stop translation

<400> 136  
Val Ala Gln Thr Arg Val Gln Trp His Asp His Ser Ser Leu Xaa Pro  
1 5 10 15  
Gln Pro His Gly Leu Asn Gln Ser Ser His Pro Ser Phe Leu Asn Ser  
20 25 30  
Gly Gly Tyr Arg His Ala Pro Pro Leu Pro Ala Asp Tyr Leu Val Phe  
35 40 45  
Cys Arg Asp Lys Val Ser His Cys Val Ala Gln Ala Gly Leu Lys Leu  
50 55 60  
Leu Asp Xaa Cys Asn Pro Pro Ser Ser Pro Ser Pro Ser Val Arg Ile  
65 70 75 80  
Thr Gly Met Ser Ser His His  
85

<210> 137  
<211> 47  
<212> PRT  
<213> Homo sapiens

<400> 137  
Arg Leu Glu Cys Asn Gly Ala Ile Ser Ala His Arg Asn Leu Arg Leu  
1 5 10 15  
Pro Gly Ser Ser Asp Ser Pro Ala Ser Ala Ser Pro Val Ala Gly Ile  
20 25 30  
Thr Gly Met Cys Thr His Ala Arg Leu Ile Leu Tyr Phe Phe Leu  
35 40 45

<210> 138  
<211> 47  
<212> PRT  
<213> Homo sapiens

<220>

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<221> SITE
<222> (44)
<223> Xaa equals stop translation

<400> 138
Arg Leu Glu Cys Ser Gly Thr Ile Thr Ala His Cys Ser Leu Asn Leu
 1              5              10              15
Thr Gly Ser Ile Asn Pro Pro Ile Pro Ala Ser Leu Ile Ala Gly Ala
              20              25              30
Thr Gly Met His His His Ser Gln Leu Ile Ile Xaa Phe Phe Val
              35              40              45

<210> 139
<211> 84
<212> PRT
<213> Homo sapiens

<400> 139
Val Thr Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Leu Gln Pro
 1              5              10              15
Leu Pro Pro Gly Phe Lys Leu Phe Ser Cys Pro Ser Leu Leu Ser Ser
              20              25              30
Trp Asp Tyr Arg Arg Pro Pro Arg Leu Ala Asn Phe Phe Val Phe Leu
              35              40              45
Val Glu Met Gly Phe Thr Met Phe Ala Arg Leu Ile Leu Ile Ser Gly
              50              55              60
Pro Cys Asp Leu Pro Ala Ser Ala Ser Gln Ser Ala Gly Ile Thr Gly
              65              70              75              80
Val Ser His His

<210> 140
<211> 86
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (15)
<223> Xaa equals stop translation

<220>
<221> SITE
<222> (67)
<223> Xaa equals stop translation

<400> 140
Val Ala Gln Thr Arg Val Gln Trp His Asp His Ser Ser Leu Xaa Pro
 1              5              10              15

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Gln Pro His Gly Leu Asn Gln Ser Ser His Pro Ser Phe Leu Asn Ser  
 20 25 30

Gly Gly Tyr Arg His Ala Pro Pro Leu Pro Ala Asp Tyr Leu Val Phe  
 35 40 45

Cys Arg Asp Lys Val Ser His Cys Val Ala Gln Ala Gly Leu Lys Leu  
 50 55 60

Leu Asp Xaa Cys Asn Pro Pro Ser Pro Ser Pro Ser Val Arg Ile  
 65 70 75 80

Thr Gly Met Ser His His  
 85

<210> 141  
 <211> 74  
 <212> PRT  
 <213> Homo sapiens

<400> 141  
 Trp Asn Gln Ala Gly Arg Cys Gly Ser Arg Leu His Phe Gly Arg Pro  
 1 5 10 15

Arg Arg Ala Asp Tyr Leu Arg Ile Gly Val Pro Asp Gln Arg Gly Gln  
 20 25 30

Arg Gly Glu Ser Pro Ser Leu Leu Lys Asn Thr Lys Ile Ser Trp Ala  
 35 40 45

Trp Trp Val Pro Val Ile Pro Ala Ile Arg Glu Gly Glu Ala Gly Glu  
 50 55 60

Ser Leu Glu Pro Gly Arg Gln Arg Leu Gln  
 65 70

<210> 142  
 <211> 77  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (12)  
 <223> Xaa equals stop translation

<220>  
 <221> SITE  
 <222> (43)  
 <223> Xaa equals stop translation

<220>  
 <221> SITE  
 <222> (76)  
 <223> Xaa equals stop translation

<400> 142



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Phe Asp Glu Leu Gly Tyr Asp Gly Ser Arg Leu Xaa Ser Gln Tyr Phe  
 1 5 10 15  
 Gly Arg Pro Arg Arg Ala Asp His Leu Arg Ser Gly Val Arg Asp Gln  
 20 25 30  
 Pro Gly Gln Arg Gly Glu Val Arg Leu Tyr Xaa Lys Tyr Lys Asn Ser  
 35 40 45  
 Trp Val Trp Trp His Ala Pro Val Ile Ala Ala Thr Gln Glu Ala Glu  
 50 55 60  
 Ala Gln Glu Ser Leu Glu Pro Gly Arg Gln Arg Xaa Gln  
 65 70 75

<210> 143  
 <211> 74  
 <212> PRT  
 <213> Homo sapiens

<400> 143  
 Trp Asn Gln Ala Gly Arg Cys Gly Ser Arg Leu His Phe Gly Arg Pro  
 1 5 10 15  
 Arg Arg Ala Asp Tyr Leu Arg Ile Gly Val Pro Asp Gln Arg Gly Gln  
 20 25 30  
 Arg Gly Glu Ser Pro Ser Leu Leu Lys Asn Thr Lys Ile Ser Trp Ala  
 35 40 45  
 Trp Trp Val Pro Val Ile Pro Ala Ile Arg Glu Gly Glu Ala Gly Glu  
 50 55 60  
 Ser Leu Glu Pro Gly Arg Gln Arg Leu Gln  
 65 70

<210> 144  
 <211> 77  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> SITE  
 <222> (12)  
 <223> Xaa equals stop translation

<220>  
 <221> SITE  
 <222> (43)  
 <223> Xaa equals stop translation

<220>  
 <221> SITE  
 <222> (76)  
 <223> Xaa equals stop translation

<400> 144

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Phe Asp Glu Leu Gly Tyr Asp Gly Ser Arg Leu Xaa Ser Gln Tyr Phe  
1 5 10 15  
Gly Arg Pro Arg Arg Ala Asp His Leu Arg Ser Gly Val Arg Asp Gln  
20 25 30  
Pro Gly Gln Arg Gly Glu Val Arg Leu Tyr Xaa Lys Tyr Lys Asn Ser  
35 40 45  
Trp Val Trp Trp His Ala Pro Val Ile Ala Ala Thr Gln Glu Ala Glu  
50 55 60  
Ala Gln Glu Ser Leu Glu Pro Gly Arg Gln Arg Xaa Gln  
65 70 75

<210> 145  
<211> 55  
<212> PRT  
<213> Homo sapiens

<400> 145  
Leu Cys Thr Leu Cys Leu Glu Glu Arg Arg His Pro Thr Ala Thr Pro  
1 5 10 15  
Cys Gly His Leu Phe Cys Trp Glu Cys Ile Thr Ala Trp Cys Ser Ser  
20 25 30  
Lys Ala Glu Cys Pro Leu Cys Arg Glu Lys Phe Pro Pro Gln Lys Leu  
35 40 45  
Ile Tyr Leu Arg His Tyr Arg  
50 55

<210> 146  
<211> 55  
<212> PRT  
<213> Homo sapiens

<400> 146  
Leu Cys Thr Leu Cys Leu Glu Glu Arg Arg His Pro Thr Ala Thr Pro  
1 5 10 15  
Cys Gly His Leu Phe Cys Trp Glu Cys Ile Thr Ala Trp Cys Ser Ser  
20 25 30  
Lys Ala Glu Cys Pro Leu Cys Arg Glu Lys Phe Pro Pro Gln Lys Leu  
35 40 45  
Ile Tyr Leu Arg His Tyr Arg  
50 55

<210> 147  
<211> 55  
<212> PRT  
<213> Homo sapiens

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&lt;400&gt; 147

Leu Cys Thr Leu Cys Leu Glu Glu Arg Arg His Pro Thr Ala Thr Pro  
1 5 10 15

Cys Gly His Leu Phe Cys Trp Glu Cys Ile Thr Ala Trp Cys Ser Ser  
20 25 30

Lys Ala Glu Cys Pro Leu Cys Arg Glu Lys Phe Pro Pro Gln Lys Leu  
35 40 45

Ile Tyr Leu Arg His Tyr Arg  
50 55

&lt;210&gt; 148

&lt;211&gt; 55

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 148

Leu Cys Thr Leu Cys Leu Glu Glu Arg Arg His Pro Thr Ala Thr Pro  
1 5 10 15

Cys Gly His Leu Phe Cys Trp Glu Cys Ile Thr Ala Trp Cys Ser Ser  
20 25 30

Lys Ala Glu Cys Pro Leu Cys Arg Glu Lys Phe Pro Pro Gln Lys Leu  
35 40 45

Ile Tyr Leu Arg His Tyr Arg  
50 55

&lt;210&gt; 149

&lt;211&gt; 59

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 149

Ile Lys Glu Leu Tyr Lys Leu Leu Glu Val Trp Ile Glu Phe Gly Arg  
1 5 10 15

Ile Lys Leu Pro Gln Gly Tyr His Pro Asn His Val Glu Glu Glu Trp  
20 25 30

Gly Lys Leu Ile Val Glu Met Leu Glu Arg Glu Lys Ser Leu Arg Pro  
35 40 45

Ala Val Glu Arg Leu Glu Leu Leu Gln Ile  
50 55

&lt;210&gt; 150

&lt;211&gt; 57

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 150

Leu Lys Glu Tyr Leu Phe Gln Val Trp Ile Glu Phe Gly Arg Ile Lys

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1 5 10 15  
Leu Pro Gln Gly Tyr His Pro Asn Asp Val Glu Glu Glu Trp Gly Lys  
20 25 30  
Leu Ile Ile Glu Met Leu Glu Arg Glu Lys Ser Leu Arg Pro Ala Val  
35 40 45  
Glu Arg Trp Val Gln Ile Leu Arg Val  
50 55

<210> 151  
<211> 59  
<212> PRT  
<213> Homo sapiens

<400> 151  
Ile Lys Glu Leu Tyr Lys Leu Leu Glu Val Trp Ile Glu Phe Gly Arg  
1 5 10 15  
Ile Lys Leu Pro Gln Gly Tyr His Pro Asn His Val Glu Glu Glu Trp  
20 25 30  
Gly Lys Leu Ile Val Glu Met Leu Glu Arg Glu Lys Ser Leu Arg Pro  
35 40 45  
Ala Val Glu Arg Leu Glu Leu Leu Leu Gln Ile  
50 55

<210> 152  
<211> 57  
<212> PRT  
<213> Homo sapiens

<400> 152  
Leu Lys Glu Tyr Leu Phe Gln Val Trp Ile Glu Phe Gly Arg Ile Lys  
1 5 10 15  
Leu Pro Gln Gly Tyr His Pro Asn Asp Val Glu Glu Glu Trp Gly Lys  
20 25 30  
Leu Ile Ile Glu Met Leu Glu Arg Glu Lys Ser Leu Arg Pro Ala Val  
35 40 45  
Glu Arg Trp Val Gln Ile Leu Arg Val  
50 55

<210> 153  
<211> 66  
<212> PRT  
<213> Homo sapiens

<400> 153  
Val Thr Gln Ala Gly Val Gln Trp Pro Asn Leu Gly Ser Leu Gln Pro  
1 5 10 15

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Leu Pro Pro Gly Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser  
 20 25 30

Trp Asp Tyr Gly His Leu Pro Pro His Pro Ala Asn Phe Cys Ile Phe  
 35 40 45

Ile Arg Gly Gly Val Ser Pro Tyr Leu Ser Gly Trp Ser Gln Thr Pro  
 50 55 60

Asp Leu  
 65

&lt;210&gt; 154

&lt;211&gt; 66

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (39)

&lt;223&gt; Xaa equals stop translation

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (49)

&lt;223&gt; Xaa equals stop translation

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (50)

&lt;223&gt; Xaa equals stop translation

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (57)

&lt;223&gt; Xaa equals stop translation

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (63)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 154

Val Ala Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Val Gln Pro  
 1 5 10 15

Leu Pro Pro Arg Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser  
 20 25 30

Trp Asp Tyr Arg His Val Xaa Pro Cys Gln Ala Asn Phe Leu Tyr Phe  
 35 40 45

Xaa Xaa Arg Glu Val Ser Pro Cys Xaa Pro Gly Trp Ser Arg Xaa Ser  
 50 55 60

Asp Leu  
 65

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72

<210> 155  
<211> 70  
<212> PRT  
<213> Homo sapiens

<400> 155  
Ser Leu Leu Leu Pro Arg Leu Glu Cys Asn Gly Ala Ile Ser Ala His  
1 5 10 15  
Arg Asn Leu Arg Leu Pro Gly Ser Ser Asp Ser Pro Ala Ser Ala Ser  
20 25 30  
Pro Val Ala Gly Ile Thr Gly Met Cys Thr His Ala Arg Leu Ile Leu  
35 40 45  
Tyr Phe Phe Leu Val Glu Met Glu Phe Leu His Val Gly Gln Ala Gly  
50 55 60  
Leu Glu Leu Pro Thr Ser  
65 70

<210> 156  
<211> 69  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (24)  
<223> Xaa equals stop translation

<400> 156  
Ser Pro Leu Leu Pro Arg Leu Glu Cys Asn Gly Val Ile Ser Ala Gln  
1 5 10 15  
Cys Asn Leu Cys Leu Pro Gly Xaa Ser Asp Ser Pro Ala Ser Ala Ser  
20 25 30  
Gln Val Ala Gly Thr Thr Gly Thr Cys Asp His Ala Arg Leu Ile Phe  
35 40 45  
Tyr Ile Phe Ser Arg Glu Arg Phe His His Val Ser Gln Asp Gly Leu  
50 55 60  
Asp Asp Leu Thr Ser  
65

<210> 157  
<211> 66  
<212> PRT  
<213> Homo sapiens

<400> 157  
Val Thr Gln Ala Gly Val Gln Trp Pro Asn Leu Gly Ser Leu Gln Pro  
1 5 10 15

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Leu Pro Pro Gly Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser  
 20 25 30

Trp Asp Tyr Gly His Leu Pro Pro His Pro Ala Asn Phe Cys Ile Phe  
 35 40 45

Ile Arg Gly Gly Val Ser Pro Tyr Leu Ser Gly Trp Ser Gln Thr Pro  
 50 55 60

Asp Leu  
 65

<210> 158

<211> 66

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (39)

<223> Xaa equals stop translation

<220>

<221> SITE

<222> (49)

<223> Xaa equals stop translation

<220>

<221> SITE

<222> (50)

<223> Xaa equals stop translation

<220>

<221> SITE

<222> (57)

<223> Xaa equals stop translation

<220>

<221> SITE

<222> (63)

<223> Xaa equals stop translation

<400> 158

Val Ala Gln Ala Gly Val Gln Trp Arg Asn Leu Gly Ser Val Gln Pro  
 1 5 10 15

Leu Pro Pro Arg Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser  
 20 25 30

Trp Asp Tyr Arg His Val Xaa Pro Cys Gln Ala Asn Phe Leu Tyr Phe  
 35 40 45

Xaa Xaa Arg Glu Val Ser Pro Cys Xaa Pro Gly Trp Ser Arg Xaa Ser  
 50 55 60

Asp Leu  
 65

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<210> 159  
<211> 70  
<212> FRT  
<213> Homo sapiens

<400> 159  
Ser Leu Leu Leu Pro Arg Leu Glu Cys Asn Gly Ala Ile Ser Ala His  
1 5 10 15  
Arg Asn Leu Arg Leu Pro Gly Ser Ser Asp Ser Pro Ala Ser Ala Ser  
20 25 30  
Pro Val Ala Gly Ile Thr Gly Met Cys Thr His Ala Arg Leu Ile Leu  
35 40 45  
Tyr Phe Phe Leu Val Glu Met Glu Phe Leu His Val Gly Gln Ala Gly  
50 55 60  
Leu Glu Leu Pro Thr Ser  
65 70

<210> 160  
<211> 69  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (24)  
<223> Xaa equals stop translation

<400> 160  
Ser Pro Leu Leu Pro Arg Leu Glu Cys Asn Gly Val Ile Ser Ala Gln  
1 5 10 15  
Cys Asn Leu Cys Leu Pro Gly Xaa Ser Asp Ser Pro Ala Ser Ala Ser  
20 25 30  
Gln Val Ala Gly Thr Thr Gly Thr Cys Asp His Ala Arg Leu Ile Phe  
35 40 45  
Tyr Ile Phe Ser Arg Glu Arg Phe His His Val Ser Gln Asp Gly Leu  
50 55 60  
Asp Asp Leu Thr Ser  
65

<210> 161  
<211> 39  
<212> PRT  
<213> Homo sapiens

<400> 161  
Glu Ser Cys Ser Val Thr Leu Ala Gly Val Gln Trp Arg Asp Leu Gly  
1 5 10 15



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75

Leu Leu Gln Pro Leu Pro Pro Lys Phe Lys Arg Phe Ser Cys Leu Ser  
20 25 30

Phe Pro Ser Ser Trp Asp Tyr  
35

&lt;210&gt; 162

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (27)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 162

Glu Ser Cys Ser Val Ala Arg Ala Gly Val Gln Trp His Asp Leu Gly  
1 5 10 15

Ser Leu Gln Pro Pro Pro Pro Arg Phe Lys Xaa Phe Ser Cys Leu Gly  
20 25 30

Leu Gln Ser Ser Trp Asp Tyr  
35

&lt;210&gt; 163

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 163

Glu Ser Cys Ser Val Thr Leu Ala Gly Val Gln Trp Arg Asp Leu Gly  
1 5 10 15

Leu Leu Gln Pro Leu Pro Pro Lys Phe Lys Arg Phe Ser Cys Leu Ser  
20 25 30

Phe Pro Ser Ser Trp Asp Tyr  
35

&lt;210&gt; 164

&lt;211&gt; 39

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (27)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 164

Glu Ser Cys Ser Val Ala Arg Ala Gly Val Gln Trp His Asp Leu Gly  
1 5 10 15

Ser Leu Gln Pro Pro Pro Pro Arg Phe Lys Xaa Phe Ser Cys Leu Gly

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                20                25                30

Leu Gln Ser Ser Trp Asp Tyr
      35

<210> 165
<211> 87
<212> PRT
<213> Homo sapiens

<400> 165
His Phe Leu Phe Ser Phe Leu Phe Phe Phe Glu Thr Gln Ser His
  1                5                10                15

Ser Val Thr Arg Leu Glu Cys Ser Gly Thr Ile Ser Ala His Cys Asn
      20                25                30

Leu Cys Leu Pro Gly Ser Ser Asn Ser Pro Ala Ser Ala Ser Arg Val
      35                40                45

Ala Gly Thr Ala Gly Thr Cys Arg Arg Ala Gln Leu Ile Phe Val Phe
      50                55                60

Leu Ala Glu Met Gly Phe His His Val Gly Arg Asp Gly Leu Asp Leu
      65                70                75                80

Asn Leu Val Ile His Pro Pro
      85

<210> 166
<211> 76
<212> PRT
<213> Homo sapiens

<220>
<221> SITE
<222> (44)
<223> Xaa equals stop translation

<220>
<221> SITE
<222> (47)
<223> Xaa equals stop translation

<400> 166
Tyr Phe His Val Ser Phe Phe Phe Phe Gly Glu Pro Arg Leu Ala
  1                5                10                15

Leu Leu Pro Arg Leu Glu Cys Ser Gly Val Ile Phe Ala His Cys Asn
      20                25                30

Leu Arg Leu Val Gly Ser Arg Glu Ser Pro Ile Xaa Ala Ser Xaa Val
      35                40                45

Ala Gly Ile Thr Val Ala Cys Gln Glu Met Gly Phe Arg His Val Asp
      50                55                60

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Gln Ala Gly Leu Glu His Leu Ala Ser Asn Asp Pro  
 65 70 75

<210> 167  
 <211> 87  
 <212> PRT  
 <213> Homo sapiens

<400> 167  
 His Phe Leu Phe Ser Phe Leu Phe Phe Phe Glu Thr Gln Ser His  
 1 5 10 15  
 Ser Val Thr Arg Leu Glu Cys Ser Gly Thr Ile Ser Ala His Cys Asn  
 20 25 30  
 Leu Cys Leu Pro Gly Ser Ser Asn Ser Pro Ala Ser Ala Ser Arg Val  
 35 40 45  
 Ala Gly Thr Ala Gly Thr Cys Arg Arg Ala Gln Leu Ile Phe Val Phe  
 50 55 60  
 Leu Ala Glu Met Gly Phe His His Val Gly Arg Asp Gly Leu Asp Leu  
 65 70 75 80  
 Asn Leu Val Ile His Pro Pro  
 85

<210> 168  
 <211> 76  
 <212> PRT  
 <213> Homo sapiens  
 <220>  
 <221> SITE  
 <222> (44)  
 <223> Xaa equals stop translation  
 <220>  
 <221> SITE  
 <222> (47)  
 <223> Xaa equals stop translation

<400> 168  
 Tyr Phe His Val Ser Phe Phe Phe Phe Phe Gly Glu Pro Arg Leu Ala  
 1 5 10 15  
 Leu Leu Pro Arg Leu Glu Cys Ser Gly Val Ile Phe Ala His Cys Asn  
 20 25 30  
 Leu Arg Leu Val Gly Ser Arg Glu Ser Pro Ile Xaa Ala Ser Xaa Val  
 35 40 45  
 Ala Gly Ile Thr Val Ala Cys Gln Glu Met Gly Phe Arg His Val Asp  
 50 55 60  
 Gln Ala Gly Leu Glu His Leu Ala Ser Asn Asp Pro  
 65 70 75

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<210> 169  
<211> 118  
<212> PRT  
<213> Homo sapiens  
  
<400> 169  
Leu Pro Arg Ile Pro Asp Cys Lys Val Pro Val Ala Arg Pro Ala Tyr  
1 5 10 15  
Lys Val Arg Asp Gln Trp Ser Asp Glu Ala Ala Arg Phe Gly Gln Asn  
20 25 30  
Asp Tyr Ile Asp Leu Leu Gly Asp Gly Ser Val His Pro Ala Gln Leu  
35 40 45  
Gln Tyr His Thr Pro Thr Trp Leu Arg Gly Phe Pro Gly Gln His Lys  
50 55 60  
Ala Asn Glu Leu Ile Lys Leu Ile His Tyr Arg Asn Leu Tyr Asp Ala  
65 70 75 80  
Lys Leu Lys Gln Asn Ser Pro Lys Arg Trp His Glu Leu Arg Lys Arg  
85 90 95  
Ile Lys Tyr Leu Met Met Gln His Asn Tyr Asn Lys Gln Asp Glu Ile  
100 105 110  
Gly Arg Glu Arg Asn Leu  
115

<210> 170  
<211> 106  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (102)  
<223> Xaa equals stop translation  
  
<400> 170  
Val Pro Arg Leu Ile Gly Ile Arg Leu Thr Leu Pro Pro Pro Lys Val  
1 5 10 15  
Val Asp Arg Trp Asn Glu Lys Arg Ala Met Phe Gly Val Tyr Asp Asn  
20 25 30  
Ile Gly Ile Leu Gly Asn Phe Glu Lys His Pro Lys Glu Leu Ile Arg  
35 40 45  
Gly Pro Ile Trp Leu Arg Gly Trp Lys Gly Asn Glu Leu Gln Arg Cys  
50 55 60  
Ile Arg Lys Arg Lys Met Val Gly Ser Arg Met Phe Ala Asp Asp Leu  
65 70 75 80

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His Asn Leu Asn Lys Arg Ile Arg Tyr Leu Tyr Lys His Phe Asn Arg  
85 90 95

His Gly Lys Phe Arg Xaa Lys Arg Lys Leu  
100 105

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<210> 171
<211> 118
<212> PRT
<213> Homo sapiens
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<400> 171  
Leu Pro Arg Ile Pro Asp Cys Lys Val Pro Val Ala Arg Pro Ala Tyr  
1 5 10 15

Lys Val Arg Asp Gln Trp Ser Asp Glu Ala Ala Arg Phe Gly Gln Asn  
20 25 30

Asp Tyr Ile Asp Leu Leu Gly Asp Gly Ser Val His Pro Ala Gln Leu  
35 40 45

Gln Tyr His Thr Pro Thr Trp Leu Arg Gly Phe Pro Gly Gln His Lys  
50 55 60

Ala Asn Glu Leu Ile Lys Leu Ile His Tyr Arg Asn Leu Tyr Asp Ala  
65 70 75 80

Lys Leu Lys Gln Asn Ser Pro Lys Arg Trp His Glu Leu Arg Lys Arg  
85 90 95

Ile Lys Tyr Leu Met Met Gln His Asn Tyr Asn Lys Gln Asp Glu Ile  
100 105 110

Gly Arg Glu Arg Asn Leu  
115

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<210> 172
<211> 106
<212> PRT
<213> Homo sapiens
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<220>  
<221> SITE  
<222> (102)  
<223> Xaa equals stop translation
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<400> 172
Val Pro Arg Leu Ile Gly Ile Arg Leu Thr Leu Pro Pro Pro Lys Val
  1             5             10             15
```

Val Asp Arg Trp Asn Glu Lys Arg Ala Met Phe Gly Val Tyr Asp Asn  
20 25 30

Ile Gly Ile Leu Gly Asn Phe Glu Lys His Pro Lys Glu Leu Ile Arg  
35 40 45

Gly Pro Ile Trp Leu Arg Gly Trp Lys Gly Asn Glu Leu Gln Arg Cys

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50 55 60  
Ile Arg Lys Arg Lys Met Val Gly Ser Arg Met Phe Ala Asp Asp Leu  
65 70 75 80  
His Asn Leu Asn Lys Arg Ile Arg Tyr Leu Tyr Lys His Phe Asn Arg  
85 90 95  
His Gly Lys Phe Arg Xaa Lys Arg Lys Leu  
100 105

<210> 173  
<211> 48  
<212> PRT  
<213> Homo sapiens

<400> 173  
Gly Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser Trp Asp Tyr  
1 5 10 15  
Gly His Leu Pro Pro His Pro Ala Asn Phe Cys Ile Phe Ile Arg Gly  
20 25 30  
Gly Val Ser Pro Tyr Leu Ser Gly Trp Ser Gln Thr Pro Asp Leu Arg  
35 40 45

<210> 174  
<211> 48  
<212> PRT  
<213> Homo sapiens

<220>  
<221> SITE  
<222> (43)  
<223> Xaa equals stop translation

<400> 174  
Gly Phe Lys Gln Phe Ser Cys Leu Ser Leu Leu Asn Ser Trp Asp Tyr  
1 5 10 15  
Arg Arg Met Pro Pro Cys Pro Ala Asn Phe Cys Ile Phe Ser Arg Asp  
20 25 30  
Gly Val Ser Pro Tyr Trp Ser Ser Trp Ser Xaa Asn Pro Asp Leu Arg  
35 40 45

<210> 175  
<211> 69  
<212> PRT  
<213> Homo sapiens

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&lt;400&gt; 175

Ala Ile Ser Ala His Arg Asn Leu Arg Leu Pro Gly Ser Ser Asp Ser  
1 5 10 15

Pro Ala Ser Ala Ser Pro Val Ala Gly Ile Thr Gly Met Cys Thr His  
20 25 30

Ala Arg Leu Ile Leu Tyr Phe Phe Leu Val Glu Met Glu Phe Leu His  
35 40 45

Val Gly Gln Ala Gly Leu Glu Leu Pro Thr Ser Asp Asp Pro Ser Val  
50 55 60

Ser Ala Ser Gln Ser  
65

&lt;210&gt; 176

&lt;211&gt; 69

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; SITE

&lt;222&gt; (23)

&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 176

Ala Thr Ser Ala Ser Arg Val Gln Ala Ile Leu Pro Gly Ser Ser Asn  
1 5 10 15

Ser Pro Val Ser Ala Ser Xaa Ile Ala Gly Thr Thr Gly Ala Cys His  
20 25 30

His Ala Gln Leu Thr Phe Val Phe Leu Val Glu Met Gly Phe Pro His  
35 40 45

Ile Gly Gln Ala Gly Leu Glu Ile Leu Thr Ser Gly Asn Pro Pro Thr  
50 55 60

Ser Ala Ser Gln Ser  
65

&lt;210&gt; 177

&lt;211&gt; 48

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 177

Gly Leu Lys Arg Phe Ser Cys Leu Ser Leu Pro Ser Ser Trp Asp Tyr  
1 5 10 15

Gly His Leu Pro Pro His Pro Ala Asn Phe Cys Ile Phe Ile Arg Gly  
20 25 30

Gly Val Ser Pro Tyr Leu Ser Gly Trp Ser Gln Thr Pro Asp Leu Arg  
35 40 45

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<210> 178  
<211> 48  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (43)  
<223> Xaa equals stop translation  
  
<400> 178  
Gly Phe Lys Gln Phe Ser Cys Leu Ser Leu Leu Asn Ser Trp Asp Tyr  
1 5 10 15  
Arg Arg Met Pro Pro Cys Pro Ala Asn Phe Cys Ile Phe Ser Arg Asp  
20 25 30  
Gly Val Ser Pro Tyr Trp Ser Ser Trp Ser Xaa Asn Pro Asp Leu Arg  
35 40 45

<210> 179  
<211> 69  
<212> PRT  
<213> Homo sapiens  
  
<400> 179  
Ala Ile Ser Ala His Arg Asn Leu Arg Leu Pro Gly Ser Ser Asp Ser  
1 5 10 15  
Pro Ala Ser Ala Ser Pro Val Ala Gly Ile Thr Gly Met Cys Thr His  
20 25 30  
Ala Arg Leu Ile Leu Tyr Phe Phe Leu Val Glu Met Glu Phe Leu His  
35 40 45  
Val Gly Gln Ala Gly Leu Glu Leu Pro Thr Ser Asp Asp Pro Ser Val  
50 55 60  
Ser Ala Ser Gln Ser  
65

<210> 180  
<211> 69  
<212> PRT  
<213> Homo sapiens  
  
<220>  
<221> SITE  
<222> (23)



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&lt;223&gt; Xaa equals stop translation

&lt;400&gt; 180

Ala Thr Ser Ala Ser Arg Val Gln Ala Ile Leu Pro Gly Ser Ser Asn  
 1 5 10 15

Ser Pro Val Ser Ala Ser Xaa Ile Ala Gly Thr Thr Gly Ala Cys His  
 20 25 30

His Ala Gln Leu Thr Phe Val Phe Leu Val Glu Met Gly Phe Pro His  
 35 40 45

Ile Gly Gln Ala Gly Leu Glu Ile Leu Thr Ser Gly Asn Pro Pro Thr  
 50 55 60

Ser Ala Ser Gln Ser  
 65

&lt;210&gt; 181

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 181

Glu Asp Phe Glu Phe Ala Leu Asp Met Thr Arg Asp Glu Tyr Asn Ala  
 1 5 10 15

Leu Pro Ala Trp Lys Gln Val Asn Leu Lys Lys Ala Lys Gly Leu Phe  
 20 25 30

&lt;210&gt; 182

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 182

Glu Asp Phe Glu Phe Ala Leu Asp Met Thr Arg Asp Glu Tyr Asn Ala  
 1 5 10 15

Leu Pro Ala Trp Lys Gln Val Asn Leu Lys Lys Ala Lys Gly Leu Phe  
 20 25 30

&lt;210&gt; 183

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 183

Glu Asp Phe Glu Phe Ala Leu Asp Met Thr Arg Asp Glu Tyr Asn Ala  
 1 5 10 15

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Leu Pro Ala Trp Lys Gln Val Asn Leu Lys Lys Ala Lys Gly Leu Phe  
20 25 30

&lt;210&gt; 184

&lt;211&gt; 32

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 184

Glu Asp Phe Glu Phe Ala Leu Asp Met Thr Arg Asp Glu Tyr Asn Ala  
1 5 10 15

Leu Pro Ala Trp Lys Gln Val Asn Leu Lys Lys Ala Lys Gly Leu Phe  
20 25 30

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/07661

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 70.1, 71.1, 71.2, 252.3, 320.1, 325, 471; 530/350; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

N\_Geneseq\_36, EST, Issued\_Patents\_NA, GenEmbl, Issued\_Patents\_AA, A\_Geneseq\_36, sptrenbl\_12, SwissProt\_38, PIR\_63,

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Database GenEmbl. LAMERDIN et al. Sequence Analysis of an 800 kb region in 19p13.2 between PDE4A and D19S584. 23 December 1998. See Sequence Comparison A.	1 ----- 7-10
X --- Y	ADAMS et al. Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library. Nature Genetics. August 1993. Vol. 4, pages 373-380, especially attached Sequence Comparison B.	1 ----- 7-10
X --- Y --- A	US 5,811,535 A (ADAMOU et al) 22 September 1998 (22/09/98), see entire document, especially column 17, line 50 to column 23, line 31 and SEQ ID NO:4 and attached Sequence Comparison C.	1 ----- 7-10, 14-15 ----- 2-6, 21

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*C* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
*D* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	*A* document member of the same patent family

Date of the actual completion of the international search 20 JUNE 2000	Date of mailing of the international search report 25 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized Signer ROBERT LANDSMAN Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07661

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WILSON, W.C. Development of a nested-PCR test based on sequence analysis of epizootic hemorrhagic disease viruses non-structural protein 1 (NS1). Virus Research. 1994. Vol. 31, pages 357-365, entire document and Sequence Comparison D.	11-12 ----- 14-16

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07661**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-12, 14-16 and 21

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/07661A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (7):

C12P 21/06, 21/04; C12N 1/20, 15/00, 15/09, 15/63, 15/70, 15/74, 5/00, 5/02; C07K 1/00, 14/00, 17/00; C07H 21/04

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

435/69.1, 70.1, 71.1, 71.2, 252.3, 320.1, 325, 471; 530/350; 536/23.5

## BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Group I, claims 1-12, 14-16 and 21, drawn to a nucleic acid, vector, host cell, method for making polypeptide and polypeptide.

Group II, claim 13, drawn to an antibody.

Group III, claim 17, drawn to a method of preventing, treating or ameliorating a medical condition.

Group IV, claims 18-19, drawn to a method of diagnosing a pathological condition.

Group V, claim 20, drawn to a method of identifying a binding partner.

Group VI, claim 22, drawn to a method of identifying an activity in a biological assay.

Group VII, claim 23, drawn to a binding partner.

The invention listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical feature for the following reasons: the special technical feature of Group I is the polypeptide and its encoding nucleic acid. The special technical feature of Group II is the antibody that binds the polypeptide of Group I, but does not share the same primary structure of the polypeptide of Group I. The special technical feature of Group III is a method of preventing a medical condition using the polypeptide or nucleic acid of Group I. The special technical feature of Group IV is a method of diagnosing a pathological condition. The special technical feature of Group V is a method of identifying a binding partner using the polypeptide of Group I. The special technical feature of Group VI is a method of identifying an activity in a biological assay using the polypeptide of Group I. The special technical feature of Group VII is a binding partner.

The special technical feature of each Group is not the same or does not correspond to the special technical feature of any other Group. The products of Group I, II and VII are structurally distinct, and the methods of Groups III-VI require different method steps and reagents for achieving different goals. The Groups are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.